Frontal Cortex Transplants and Enriched Postoperative Environments Promote Recovery of Function From Sensorimotor Cortex Injury.

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Abstract

E20 foetal frontal cortex tissue was transplanted as a suspension into the parenchyma adjacent to a sensorimotor lesion cavity made 30 days previously in the right hemisphere of adult rats. Immediately after transplant surgery the rats were placed in one of two postlesion housing conditions, 'enriched' or 'standard', and their postoperative performance was quantified using a beam running task that they had been trained on prior to lesion surgery. The lesion animals were impaired in comparison to the controls as measured by foot faults, but not as measured by running times. Rats that received transplants were initially less impaired in comparison to their nontransplanted counterparts. Rats housed in the enriched environment were usually less impaired than their standard-housed counterparts, but error frequency was not influenced by environmental condition and the enriched rats made significantly more minor foot faults at the first postransplant test session, but thereafter the enriched rats made significantly less major foot faults than the standard housing rats. The combination of transplant and enriched environment failed to result in greater recovery than either treatment in isolation. Histological analysis revealed that 12 of 13 transplants survived and that the transplant volumes of the enriched and standard rats were not significantly different.

1.0 Introduction

While a degree of spontaneous recovery may occur following brain damage in mammals, central nervous system neurones, once lost, are not replaced. Consequently the prognosis for most forms of neurological damage is poor. Within this context the observation that transplanted foetal nervous tissue not only survives, grows and forms axonal connections with the host brain but also, in some cases, ameliorates functional impairment in brain damaged rats has attracted considerable attention. Indeed numerous studies have demonstrated that such transplants are capable of enhancing recovery of function from brain damage (Björklund and Stenevi, 1985; Dunnett and Richards, 1990). However the majority of such studies are concerned with modelling neurodegenerative damage to subcortical regions. Relatively few studies have examined a transplants ability to enhance recovery from acute brain damage to the cortex, and especially the sensorimotor cortex. Moreover those studies that do examine transplants in the context of cortex lesions commonly report problems with both transplant survival and obtaining any functional benefits from the transplant (Slavin et al, 1988; Swenson, Danielson, Klausen, Erlich, Zimmer and Castro, 1989; Dunnett, Ryan, Levin, Reynolds and Bunch 1987).

This study was designed to establish: 1) that a suspension transplant of frontal cortex tissue can survive when grafted into a lesioned sensorimotor cortex, 2) that such transplants will enhance recovery of function to a greater extent than any spontaneous recovery that may be experienced and 3) that enriched postoperative housing will similarly enhance recovery and may potentiate transplant enhanced recovery.

1.1 SENSORIMOTOR CORTEX LESIONS

Feeney, Gonzalez and Law (1982) justify the experimental examination of sensorimotor cortex [SMC] lesions by stating that behavioural deficits resulting from damage to the motor cortex in humans 'can persist indefinitely' and that 'despite major advances in the understanding of brain function, no medical treatments have been developed to promote recovery from brain damage.' However studies of transplants made into this area provide mixed, and sometimes contradictory, evidence as to the ability of a transplant to enhance recovery of function. Thus a re-examination of the issues is warranted, in order to identify the precise manipulation of influential variables that would help to maximise a transplants potential for enhancing recovery of function.

The behavioural consequences of damage to the sensorimotor cortex are well documented. A survey of the relevant literature reveals consistent behavioural impairment of motor behaviour as a result of experimental damage to the sensorimotor cortex. Those studies that employ such a lesion and then observe its behavioural consequences on a rats ability to traverse a narrow beam find the rats are (initially) severely impaired. They run slower, make more foot faults and usually display an aberrant movement pattern (Davis, Edgards, Crisostomo, Duncan, Propst and Feeney, 1978; Feeney et al, 1982; Goldstein and Davis, 1990 Gentile, Green, Nieburgs, Schmelzer and Stein, 1978; Held, Gordon and Gentile, 1985 Slavin et al, 1988; Swenson et al, 1989). Kolb and Whishaw (1983) conclude by stating that 'it is now established that ablation of the motor cortex in rodents produces severe

disruptions in discrete digit movements, forelimb movements, tongue extension and manipulation, and certain postural reflexes'.

However sensorimotor cortex injury is not permanently disabling in the rat. Of the studies listed above all report some form of spontaneous behavioural recovery after cortex lesions. Nevertheless such spontaneous recovery is usually only partial as it seldom results in behaviour commensurate with nonlesioned animals. Furthermore, those animals that do exhibit complete recovery in terms of speed and foot faults continue to display an aberrant movement pattern as measured by high speed film analysis (Gentile et al, 1978 Held et al, 1985 Slavin et al, 1988).

While the studies listed above report very similar impairments as a result of cortical lesions, the actual lesions, employed by the different studies vary. Davis et al (1978), Feeney et al (1982) and Swenson et al (1989) employed unilateral lesions while the remainder of the studies listed above used bilateral lesions. Davis et al employed a unilateral lesion in order to model 'the effects of various therapies on recovery of motor function after stroke', similarly Feeney et al employed a unilateral lesion as they were interested in modelling the 'unilateral damage to the motor cortex'. Because of this Davis et al, Feeney et al and Swenson et al used narrow beams (1.9cm, 2.5cm and 2.5 cm, respectively) while the other studies above employed relatively wide beams (5cm). It would seem likely that while a bilaterally lesioned animal is capable of traversing the wider beam (albeit with difficulty) it would be incapable of traversing a narrower beam. Whereas a unilaterally lesioned animal would have very little difficulty traversing the wider beam, it would display considerable functional impairment traversing the narrower

beam. Thus the use of a unilateral lesion in conjunction with a narrow beam is comparable to the use of a bilateral lesion in conjunction with a wider beam.

1.2 TRANSPLANTS

In the context of sensorimotor cortex lesions, there is contradictory evidence as to a transplants ability to enhance recovery of function. The most behaviourally successful findings were reported by Plumet, Cadusseau and Roger (1991) who transplanted E16 tissue drawn from the 'rostal 3rd. of the cortex' into the unilateraly lesioned frontal cortex (including the anterior SMC) of neonatal rats, half of which received a transplant of solid tissue, the other half a transplant of suspended tissue, immediately after lesion surgery. They reported a 100% survival rate for the transplants and found that the transplants induced partial reduction of motor impairment (paw reaching). They observed that while lesions impaired the performance of a skilled forelimb task the grafted groups were recovered compared to lesion only animals. The grafted animals also experienced almost complete sparing on some behaviours, as their rate of successful paw reaches, reaching attempts and grasping attempts were not significantly different to controls.

Sandor, Gonzalez, Moseley and Sharp (1991) grafted E17-18 frontal cortex tissue into a unilateral SMC lesion cavity made 7 days previously, when the rats were neonates. The mature rats then underwent training on a lever pressing task, and once they had reached criterion the transplants were removed and they were retested. The authors conclude that as the removal of the transplants resulted in a significant deterioration of motor performance, previously shown to be typical of

SMC lesions, the transplants 'functioned in a way analogous to [the] normal [sensorimotor] cortex'. While the authors also note that not all animals showed impaired motor behaviour after transplant removals, they suggest that this was due to the imperfect removal of the transplants (by both surgery and immunological rejection), and thus the residual tissue continued to function as the SMC. However the authors did not include a lesion-only group in the study, preferring instead to compare the functional impairment produced by the transplants removal to the impairment observed in a previous study that examined the functional consequences of a SMC removal (Gonzalez, Poncelet, Loken and Sharp, 1986) and arrived at their conclusions on the basis of this comparison.

In a less successful study concerned with examining the behavioural consequences of transplants into the lesioned sensorimotor cortex Slavin et al (1988) studied beam-running performance but only found very limited transplant benefit and only then with the additional co-treatment of Gm1 gangliosides. The authors transplanted one of E15, 17 or 19 sensorimotor cortex material or E19 frontal cortex tissue into a bilateral sensorimotor cortex lesion, with or without the addition of Gm1 gangliosides injected for 14 days after grafting. All the different transplant types failed to elicit any sparing of function in the absence of Gm1 gangliosides, and the animals that received Gm1 alone performed worse than the lesion-only animals. Furthermore all transplanted animals performed worse than control animals, as measured by running time and foot errors. Moreover the animals that received the E19 frontal cortex grafts (without GM1 ganglioside injections) also displayed an aberrant motor behaviour pattern. Only the transplant plus ganglioside group had running times which were not significantly different from controls Even so the combination group continued to display an aberrant motor

behaviour pattern. All groups experienced poor rates of transplant survival (30-40%) and those transplants that did survive were smaller than expected.

In a study with no transplant induced behavioural benefit Swenson et al (1989) transplanted solid E14/15 presumptive sensorimotor cortex tissue into a unilateral frontal cortex (including the anterior SMC) lesion cavity in neonates. Of the 13 transplants made 12 survived, with 6 demonstrating good growth and integration into the surrounding tissue (though no check of the degree of the appropriateness of the integration was made). However 5 'overgrew' the lesion cavity and very likely projected into the striatum causing additional damage, and 1 animal had a transplant that was small and poorly connected. All lesioned animals were impaired in comparison to controls and lesion only animals as hindlimb foot faults made while performing a beam running task were not improved by a transplant, and the transplanted animals retained the lesion induced behaviour pattern (The contralateral limb was more impaired than the ipsilateral limb). However the authors do report that in previous studies of a similar design they observed the transplants to have developed connections 'characteristic of [the] motor cortex'. Unfortunately no attempt to replicate such findings were made in their 1989 study.

1.3 Enriched Environments as a Therapy for Cortical Brain Damage.

Another common approach to ameliorating behavioural dysfunction resulting from brain damage is the use of an enriched postoperative environment. A number of review articles have all reached the same conclusion, that an enriched postoperative environment is

beneficial for functional recovery after cortical injury (Dalrymple-Alford and Kelche, 1985; Davis et al, 1978; Finger and Stein, 1982; Will and Kelche, 1992). In the most recent review Will and Kelche conclude by stating that enriched postoperative environments 'constitute a... powerful "therapeutic tool".... of high efficacy and certainly low risk'. Likewise Finger and Stein conclude their chapter on 'Environmental and Experiential Determinants of Recovery of Function' by stating 'environmental enrichment.... must be given serious consideration in this context [recovery from brain lesions]', having commented previously, 'findings support the contention that postoperative enrichment can facilitate recovery from brain damage'.

Two studies that specifically examined the benefit of a postoperatively enriched environment for recovery of impaired locomotor behaviour come to similar conclusions. In their 1985 article Held et al examined the role of enriched environments for the recovery of behavioural impairment, resulting from a sensorimotor cortex lesion, as observed on a beam running task. They comment that 'Exposure to an [enriched] environment.... reduced the initial deficit in locomotor performance following removal of the sensorimotor cortex, and facilitated the recovery following such damage' as measured by beam running speed.

In a similar vein Kolb and Gibb (1991, in Will and Kelche, 1992) examined postoperative enrichment in the context of beam running [likely in the context of a sensorimotor cortex lesion, but it is not stated], and found that, for both bilateral and unilateral damage, the postoperatively enriched subjects did not make significantly more rear foot faults, while running on the beam, than controls.

Davis et al (1978) have shown that a functional benefit derived from a pharmacological agent (amphetamine), after unilateral SMC injury, is dependent upon the animals being allowed to walk (be it during performance of a beam task or just general locomotion in their cages, such activity being analogous with general ambulatory behaviour within an enriched environment) during amphetamine intoxication. When the animals were restrained from walking during amphetamine intoxication they failed to display any amphetamine induced improvement in recovery. Furthermore any deleterious effect of a pharmacological agent (haloperidol) is also dependent on experience, as those animals that received haloperidol and then performed the beam task showed a 'dramatic reduction in the rate of recovery', whereas those animals that were given haloperidol and were also restrained from walking had the same rate of recovery as lesion-only animals.

Despite these positive results very few attempts have been made to examine any possible interaction between an enriched environment and neural transplants, even in light of such results as Held et al (1985). Moreover it has been previously noted the use of other therapies in addition to a transplant often produces greater benefit than a transplant alone. One such addition is the use of Gm1 gangliosides to enhance graft induced recovery of function, as previously mentioned per Slavin et al (1988). While Slavin et al found a limited behavioural benefit Lescaudron and Stein (1990) found a strong benefit from the addition of Gm1 gangliosides to a frontal cortex transplant made into the lesioned occipital cortex. The animals with both the transplant and the gangliosides showed greater functional recovery than the animals with either transplants or ganglioside treatment alone.

Therefore, in light of the fact that an enriched environment is an effective therapy in its own right for SMC injury, and that transplant induced recovery can benefit from additional treatments, it would seem reasonable to examine whether the combination of transplant and enriched environment would produce a combination benefit in the lesioned SMC. In their transplant review article Cassel et al (1992) state, in the context of combining transplants and enriched environment, that the 'physical and social enrichment of the postoperative environmental conditions would constitute an additional tool to further enhance the probability of increasing the beneficial effects of grafts'.

To date only 2 studies of this type have been reported. Dunnett, Whishaw, Bunch and Fine (1986) transplanted CRL 15/16mm (E16) ventral forebrain tissue, in suspension, into the ipsilateral dorsolateral frontal cortex, which had been deaffernated by a nucleus basalis lesion 7 days before. Thereafter half the animals were placed into an enriched environment and half into an 'impoverished' laboratory environment (specifically designed to 'reduce coordinated paw activity and manipulation movements of the mouth', otherwise typical of standard laboratory housing). While no behavioural assessment was made the animals were sacrificed at 4 or 10 weeks and an histological examination performed. At 4 weeks post-transplant the enriched subjects showed significantly greater fibre outgrowth than the impoverished subjects, but this advantage had disappeared by 10 weeks at which time both groups had significantly greater fibre outgrowth than any group at 4 weeks.

The other combination study (Kelche, Dalrymple-Alford and Will, 1987) housed subjects in either an enriched or standard condition

after transplanting solid septal material into a fimbria-fornix lesion cavity. The animals were then tested at 2 and 10 months post-transplant on the Hebb-Williams maze. At 10 months the enriched animals made significantly fewer initial errors. However all lesioned animals remained impaired in comparison to sham-operated animals. Nevertheless the combined treatments significantly attenuated the lesion induced impairment, something which neither the transplant nor the enriched environment did alone.

Although neither Swenson et al (1989) nor Slavin et al (1988) found any transplant benefit for beam running in the context of a unilateral or bilateral (respectively) SMC lesion, it is worth revisiting the issue to determine whether a transplant in combination with an enriched preoperative environment may be better capable of promoting behavioural recovery from a unilateral SMC lesion.

1.4 THE PRESENT STUDY DESIGN.

The present study examined the influence of a neural transplant and a postoperative enriched environments on the behavioural consequence of a sensorimotor cortex lesion, measured by beam running. As discussed above SMC lesions can be expected to result in functional impairment upon beam running task, specifically it is predicted the lesioned animals will (initially) run slower, and make more foot errors, than the control animals. These predictions are based upon the results of Davis et al (1978), Feeney et al (1982), Gentile et al (1978), Slavin et al (1988) and Swenson et al (1989), all of whom reported similar functional impairment in rats with SMC lesions when performing a beam running task. In order to ensure that the appropriate cortical areas for such an

impairment were lesioned an examination of a number of microstimulation studies and neural atlases (Hall and Lindholm, 1975; Hicks and D'Amato, 1975; Neafsey and Sievert, 1982; Neafsey et al, 1986 and Zilles, 1985) was made and the lesion site determined as a result (See section 2.3.1).

The present study employed a lesion-transplant delay of 30 days. This was in direct contradiction to the prevailing view in the literature that transplant survival (and to a lesser extent functional recovery) is greatly promoted by transplanting within the critical delay period of seven to ten days after lesioning. The reasons for choosing such a long postlesion delay are threefold. There is growing evidence that the critical delay period may not always be beneficial for transplant survival, nor necessarily beneficial for promoting functional recovery. In the context of transplant survival Dunnett, et al (1987 experiment 4), Lescaudron and Stein (1990), Soares and McIntosh (1991), Stein, Palatucci, Kahn and Labbe (1988) and Swenson et al (1989) report that a delay of 7 days resulted in good survival (including some instances of 100% survival), Slavin et al transplanted after a 7 day delay and reported that transplant survival was uniformly poor (30-40%). Furthermore Dunnett et al (1987 experiments 1-3), Justice, Moran, Deckel and Robinson (1989), Plummet et al (1991) and Swenson et al (1989), all employed delays outside the window, ranging from none to 6 weeks, and reported good transplant survival, including instances of 100% survival. Moreover, Soares et al, who specifically examined different delay periods, reported that delays of up to and including 2 weeks resulted in viable (surviving) parietal cortex transplants. Only those transplants made 4 weeks after lesioning were nonviable. Furthermore, in their 1985 study Cotman, Nieto-Sampedro and Whittmore comment that the survival rates of

striatal tissue grafted into the lesioned cortex showed 'significant improvement... as long as 20 days after surgery'.

In the context of functional recovery and the critical delay period Soares et al (1991), Dunnett et al (1987 experiment 4), Stein et al (1988) and Lescaudron and Stein (1990) all report transplant induced functional recovery when transplanting within the critical delay period. However Slavin et al (1988) stated that in two different experiments, both using a 7 day delay, that transplants were either ineffective or deleterious. Moreover Plummet et al (1991) transplanted immediately after lesioning and reported partial enhancement of recovery.

There is also a general consensus within the literature that transplant survival maybe promoted by host derived trophic support while transplant induced recovery is promoted by donor supplied trophic support (Cotman et al, 1985; Kimble, 1990; Lescaudron and Stein, 1990 and Dunnett et al, 1987). Moreover we know that endogenous levels of trophic factors are peaking within the critical seven to ten day delay period (Nieto-Sampedro, 1982). Furthermore there is evidence that foetal frontal cortex tissue is rich in trophic factors (Stein et al, 1985) and that such transplant supplied trophic factors rescue axotomized neurons (Kimble), even when transplanted in an heteretopic manner (Lescaudron and Stein, 1990 and Swenson et al, 1989). Therefore, in order to examine the functional benefits of donor supplied trophic factors alone it was decided to transplant foetal frontal cortex tissue 30 days after the lesion when levels of endogenous trophic factors had returned to normal. Thus, in the absence of appropriate reinnervation, any transplant survival and functional recovery induced by the transplant would very likely be due to the trophic support supplied by the frontal cortex material.

The fact that Slavin et al (1988) reported no benefit from transplanting E19 frontal cortex material may suggest that to use frontal cortex tissue in such a manner would be inadvisable. However the author feels that it is worthwhile revisiting the issue especially in light of the fact that a number other studies have reported very positive results from using such material (Lescaudron and Stein, 1990; Plumet et al, 1991; Sandor et al, 1991; Soares et al, 1991 and Stein et al 1985). Furthermore this study employed a different form of frontal cortex transplant, a suspension, graft, which was expected to enhance the ability of the material to promote functional recovery of function.

Complimentary to the contention that transplanting in the 7-10 day window is advisable there is also a contention that younger (<E18) foetal material is desirable, as it is supposedly better able of enhancing recovery of function than older foetal material (Slavin et al, 1988 and Kimble, 1990). However there is also evidence against this notion, Dunnett, et al (1987), Lescaudron and Stein (1990), Slavin et al (1988) and Stein et al (1988) all employ older (E18+) tissue and report some form of transplant benefit for functional recovery. But Dunnett et al, Justice et al (1989) and Slavin et al used younger (<E18) material and report a behaviourally deleterious transplant influence and Dunnett et al, Slavin et al, Soares et al (1991) and Swenson et al (1989) all report no functional benefit, using <E18 tissue. Dunnett et al commented that 'the surviving grafts of younger embryonic origin [E16] had no detectable functional effect'. However an examination of the effect of transplant age upon transplant survival reveals the opposite, Dunnett et al , Slavin et al and Stein et al employed E18+ material and report poor transplant survival. While Dunnett et al, Justice et al, Plumet et al (1991), Senatorov, Obuhova

and Fülöp (1991), Soares et al and Swenson et al all report good survival when using younger tissue.

As the purpose of the present study was to promote recovery of function, as opposed to promoting transplant survival per se, it was decided to employ relatively older foetal tissue of an age that had previously been found to be beneficial for behavioural recovery (E20, Labbe, Firl, Mufson and Stein, 1983 and Dunnett et al, 1987).

As the variation of something as basic as the age of the transplant can yield improved recovery it would seem reasonable to examine other such factors to see if doing so might bring like effects. One such factor is the method tissue preparation, employing either a solid or suspension transplant. Due to its unique nature the suspension transplant seems ideally capable of maximising the rapid supply of trophic substances to the damaged brain. As noted by many authors (Kimble, 1990; Lescaudron and Stein, 1990; Plumet et al 1991 and Soares et al, 1991) lesions and transplants commonly produce gliosis which separate the host-donor tissue at their interface, which can reasonably be expected to inhibit the transplant's survival and/or its ability to exert a beneficial influence. Furthermore when discussing what is required for a transplant to survive and influence the host Kimble comments 'to survive, graft tissue must establish connections with the host blood supply within a short period of time' and Cotman et al (1985) state that transplant survival 'depends on oxygen and nutrient supply'. If transplants require rapid access to host blood supply, the consequent nutrients, the damaged and at risk neurons and the ability to pass through a gliotic barrier, it would seem useful to employ a suspension transplant. Firstly because a suspension transplants is less neurologically traumatic than a solid

transplant. Secondly, a suspension transplant may be easily placed in the parenchyma and thus it is instantly exposed to a nurturing infrastructure, allowing it immediate access to the required blood and nutrient supply, and the ability to exert an influence upon the host, as soon as it is transplanted. Third, by transplanting into the parenchyma the transplant avoids the problems associated with the negative neural phenomena (*ie.* glial scarring, toxic substances per Cotman et al, 1985) present in the lesion cavity.

There is considerable support for the use of suspension transplants, Cassel, Kelche, Majchrzack and Will, (1992) conclude in their review article that 'All [transplants structural and/or functional] mechanisms summarised... except E [providing a 'regeneration bridge'], can be provided by intraparenchymal suspension grafts'. Furthermore Cassel et al comment that intraparenchymal grafting of 'cell suspensions is probably the grafting technique with the largest application possibilities, since it allows precise implantation... with very limited damage to the host'.

The only study to have examined the differences between suspension and solid transplants in the sensorimotor cortex concluded that there was no difference between the two, on both histological and behavioural measures (suspension placed in the parenchyma, solid into the lesion cavity), Plumet et al (1991). Furthermore they reported a 100% transplant survival rate, in comparison to Slavin et al's (1988) survival rate of 30-40%, who only grafted solid frontal cortex transplants into the SMC. Why Plumet et al obtained such good solid transplant survival when Slavin et al did not is possibly because Slavin et al's frontal cortex transplants were relatively old, E19, whereas Plumet et al's were younger,

E16, and thus better able to survive. In light of the points made above and especially the comment made by Slavin et al, that their poor rate of transplant survival was likely due to the possibility that the lesioned 'sensorimotor cortex may not provide a sufficient physiological or structural matrix for transplant attachment and growth', this study employed suspension transplants made into the parenchyma adjacent to the lesion.

1.5 SUMMARY

In summation the present study examined the ability of E20 suspension transplants made of frontal cortex tissue, in combination with postoperative enriched environments, to ameliorate the behavioural impairment resulting from a unilateral SMC lesion displayed by rats performing a beam running task. It was expected that the rats would be(initially) slower on the beam, and make more foot faults, than the nonlesioned rats. It was also expected that the transplants would survive and grow well. Furthermore those animals with a transplant or housed in an enriched environment would experience a greater degree of recovery of function than those animals without a transplant or housed in a standard environment, and that the transplant and enriched environment, in combination, would promote recovery of function to a greater extent than either therapy by itself.

2.0 Methods.

2.1 Subjects.

The fifty Sprague-Dawnley female rats, bred in the animal facility at the department, were 170 days old at the start of training. Before transplant surgery the rats were housed in standard laboratory cages, 3-4 per cage, 24 hours after transplant surgery they were housed either in standard cages or in an enriched environment cage according to their allocated experimental housing condition. The colony was lighted on a 12 : 12hr. inverted light-dark cycle with tests during the dark part of the cycle. After each daily session the rats were fed a restricted diet to maintain 85% of *ad libitum* body weight throughout training, water was always available *ad libitum* .

2.2 Apparatus and Task

2.2.1 Environments.

The standard laboratory cages contained 3-4 rats and were plastic, 18.5cm high x 51cm long x 33.5cm wide, with a wire mesh roof. The two, identical, enriched environment cages were tin metal, 45cm high x 45 cm long x 100cm wide, with a wire mesh front and roof, and each housed 9 rats (the first contained 4 transplanted and 5 nontransplanted rats and *vice versa* for the second cage). The enriched environment cages contained a number of junk objects with which the rats could interact (plastic plumbing pipes, golf balls, iron chains, wooden blocks), changed twice a week as per Dunnett et al (1986). These objects were chosen to

ensure that their manipulation did not provide specific training of those motor skills that were relevant to the behavioural test. The enriched rats were transferred to 4 standard cages during testing (typically 3-4 hours) and then returned to the enriched environment.

2.2.2 LOCOMOTOR TASK

All rats were trained preoperatively to cross a narrow elevated beam (Figure 1) and were observed from the left side (contralateral to the lesion). The wooden beam was similar to that used by Held et al (1985), raised 25cm above a table that was covered with thick foam rubber, and positioned between two sets of photoelectric cells used to record the time taken to traverse the narrow 200cm runway. An initial training beam was 5 cm. wide while the actual training/testing beam was 2.5 cm wide. The narrower beam is suitable for rats with a unilateral motor cortex lesion (Davis et al, 1978; Feeney et al, 1982: Gentile et al, 1978 and Swenson et al, 1989). The 20cm, x 5cm start platform tapered towards the beam. The metal goal box at the other end was enclosed to provide a semidark environment, into which was placed the reward, a 01).g piece of chocolate. The beam was marked with 4 horizontal lines along its complete length to record the various types of foot faults/errors. The vertical distance between each line was 15mm, with the top line 5mm below the surface of the beam. The high level of light (see below) necessary for filming was used throughout all training and testing sessions. The animals adjusted quickly and with little difficulty to the high light levels.

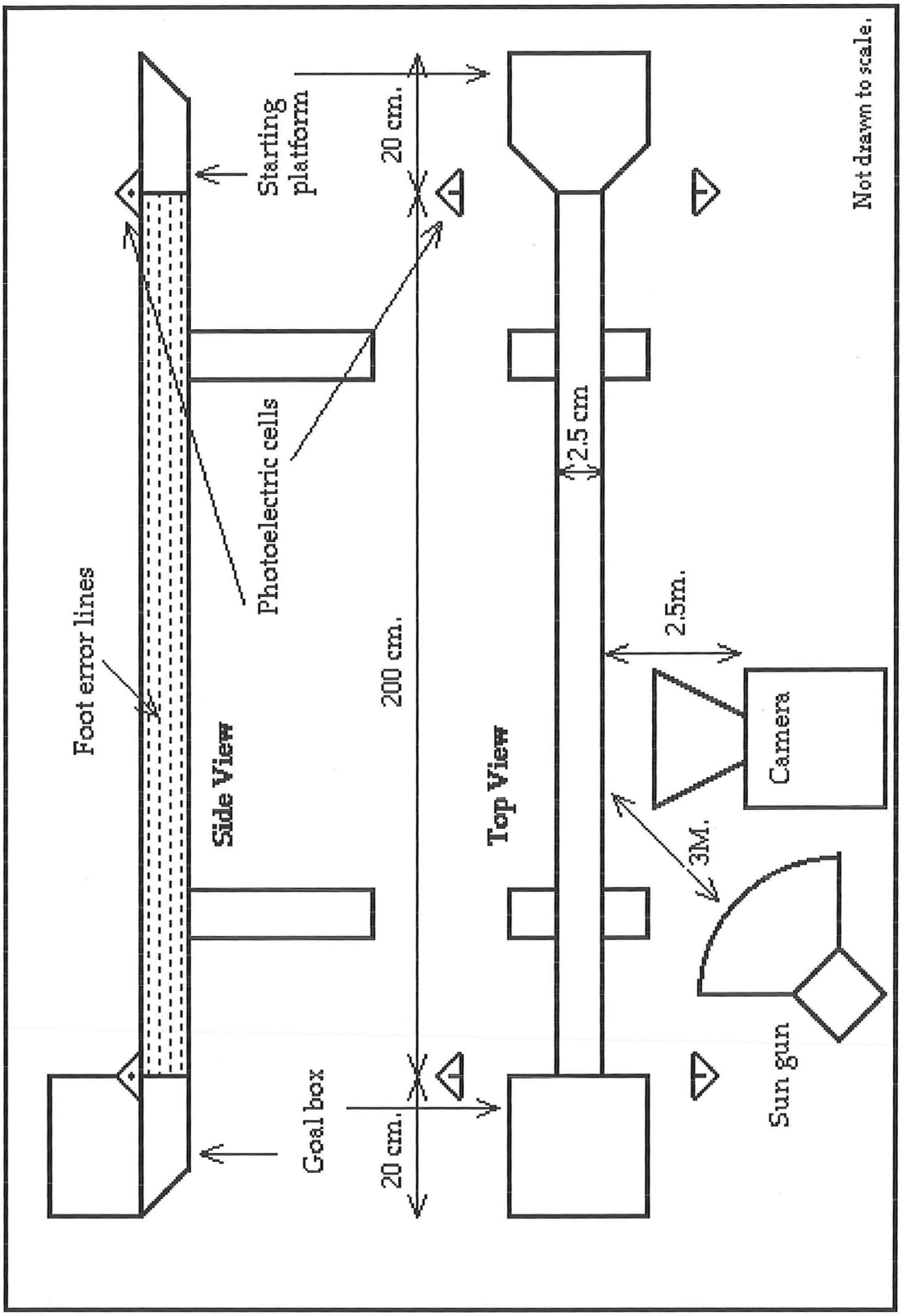

Figure 1 Schematic representation of the beam task apparatus.

2.2.3 CINEMATOGRAPHIC EQUIPMENT

A tripod-stabilised spring-driven movie camera (Bolex H 16 Reflex equipped with a Berthiot Paris PanCinor 1:2.4 lens) was used with Eastman Ektachrome High Speed 7250 Tungsten film. The camera was placed perpendicular to the runway at a distance of 2.5 metres, which provided a lateral view of the rat's movement. The surface of the runway was used as a horizontal reference. A 3 metre tape measure was attached to the side of the beam as a reference for horizontal displacement. For maximum contrast between the rat and the beam a black background was placed behind the beam. The timer was placed within camera shot and a card indicating subject number, session number and date was also within shot. A sun gun was used to provide filming light, placed behind and to the left of the camera. Frame-by-frame analysis was done on a custom editing machine.

2.3 PROCEDURE

2.3.1 SURGERY.

LESION SURGERY. The appropriate co-ordinates for the lesion site were found by consulting a number of neurological atlases and microstimulation studies (Hall and Lindholm 1974; Hicks and D'Amato 1975; Neafsey et al, 1982, Neafsey et al, 1986 and Zilles 1985). From each a neural 'map' of the sensorimotor cortex (including the fore and hind limb control areas) was generated. Thereafter the different maps were scaled to equivalence and collapsed upon each other (Figure 2). The outer boundary of the resulting composite map was taken to represent the outer extent of the sensorimotor cortex. The lesion area (Figure 3) was

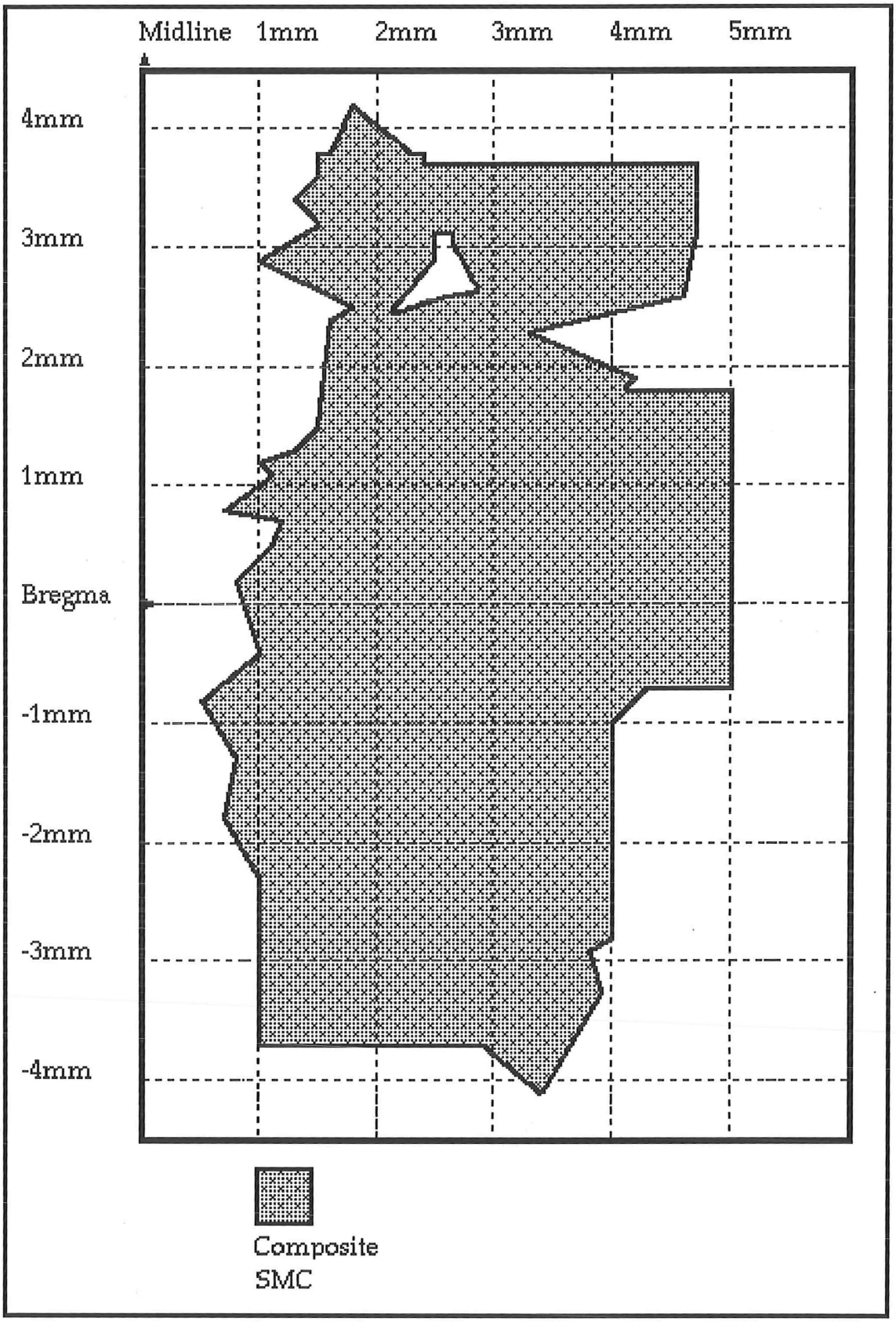

Figure 2. Composite Sensorimotor Cortex, as derived from anatomical and microstimulation studies.

determined as that part which covered as most of the composite map so as to include the primary motor cortex, the forelimb motor cortex and the hind limb motor cortex. and the central region where there was unanimous agreement between the studies as to the regions function. Figure 4 shows the composite map of the sensorimotor cortex with the lesion area superimposed on it.

The rats were anaesthetised with a mixture of Ketamine (100mg/ml/kg) and Xylazine (40mg/ml/kg). Unilateral (right hemisphere) cortical lesions were made by aspiration using standard stereotaxic techniques. The skull above the lesion site was removed and the lesion area was demarcated by cutting the cortex to a depth of 2 mm using a microscalpel. The lesion area was then cut in a criss-cross fashion and the tissue removed by gentle aspiration. As can be seen from Figure 5, the lesion covers the majority (73.2%) of the primary cortex identified as responsible for fore and hind limb locomotor behaviour. After surgery the animal was removed from the stereotaxic apparatus, the wound was cleaned and sutured, and an antibiotic cream (neomycin) applied the animal was then placed in an individual cage with food and water available *ad libitum* . Control rats (n=11) received anaesthesia and had their scalps cut and sutured.

TRANSPLANT SURGERY. The dams were deeply anaesthetised with an overdose of Nembutal on the 20th. day of gestation (CRL 23/24mm) and a midline peritoneal incision was made to expose the uterus containing the embryos. The foetuses were individually removed and placed on a sterile slide (with sterile 0.6% glucose saline) and the foetal brains removed under a dissecting microscope. The graft tissue was obtained from the frontal cortex (Figure 4). The olfactory bulbs were cut

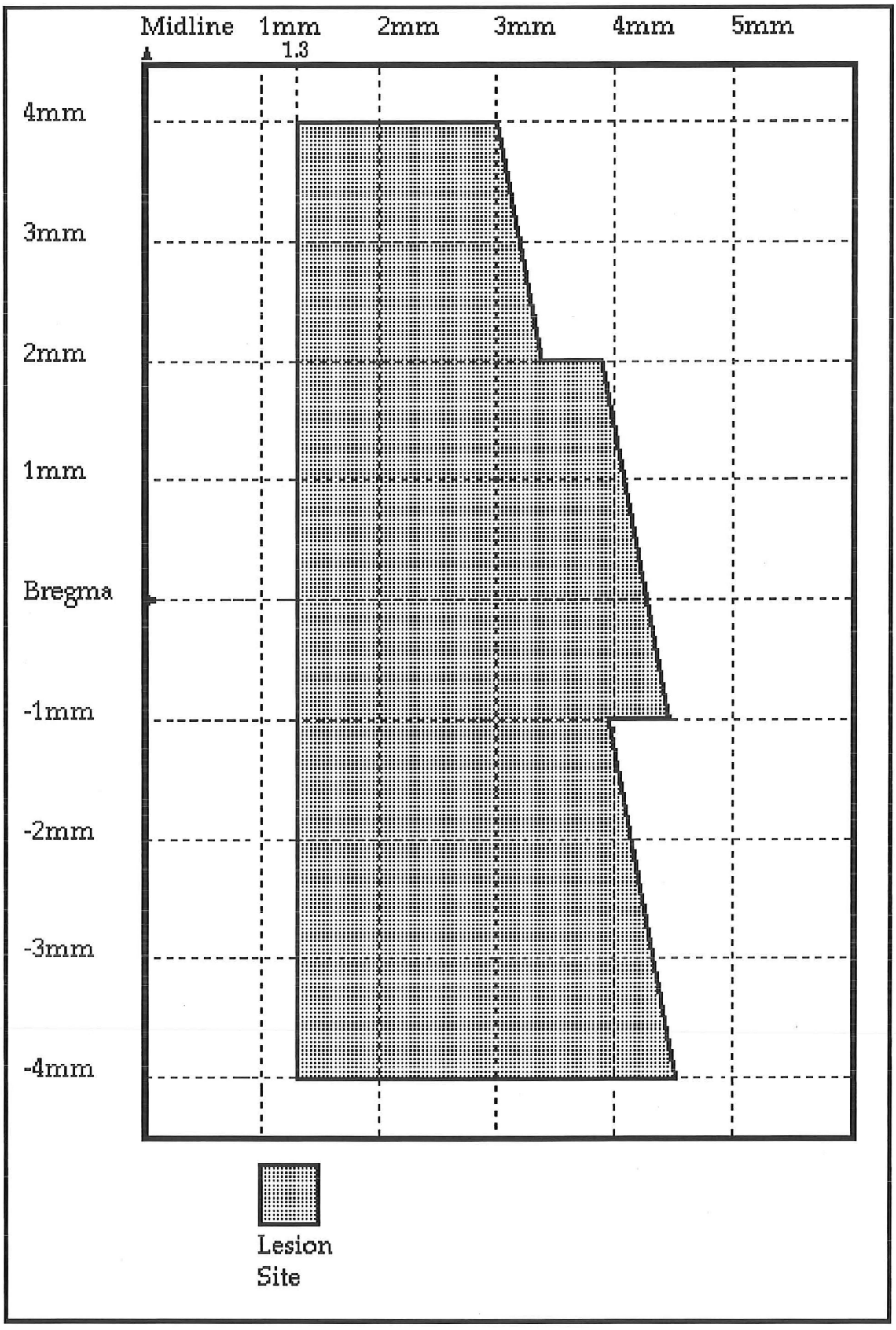

Figure 3. The present study's lesion site

away at their base and the frontal pole dissected by a coronal cut made immediately anterior to the septal region. The frontal pole tissue was then transferred to a fresh solution of sterile 0.6-glucose saline. This procedure was repeated until sufficient material had been gathered, typically 12-14 embryos.

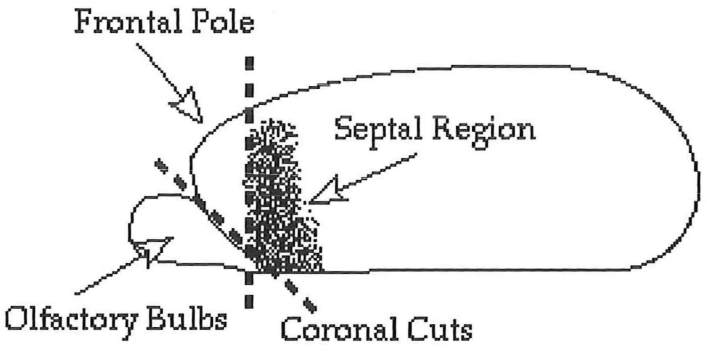

Figure 4, Schematic representation of transplant tissue origin (Lateral view).

The tissue was then disassociated by trypsination (0.1% trypsin [sigma grade II] in glucose saline) in a water bath, maintained at 27 degrees Celsius, for 20 minutes. Immediately afterwards it was repeatedly rinsed with glucose saline to remove any trace of trypsin. The suspension mixture was made up to its desired volume (one piece per 10µ glucose saline) by adding glucose saline. Thereafter a cell suspension was made using a fire-polished Pasteur pipette, as per Björklund et al (1985). The procedure with the pipette was repeated once more during the transplant operations to ensure the tissue remained evenly suspended. As each subject was readied 8µl of suspended tissue was drawn up into a 10µl Hamilton micro-syringe in preparation for injection.

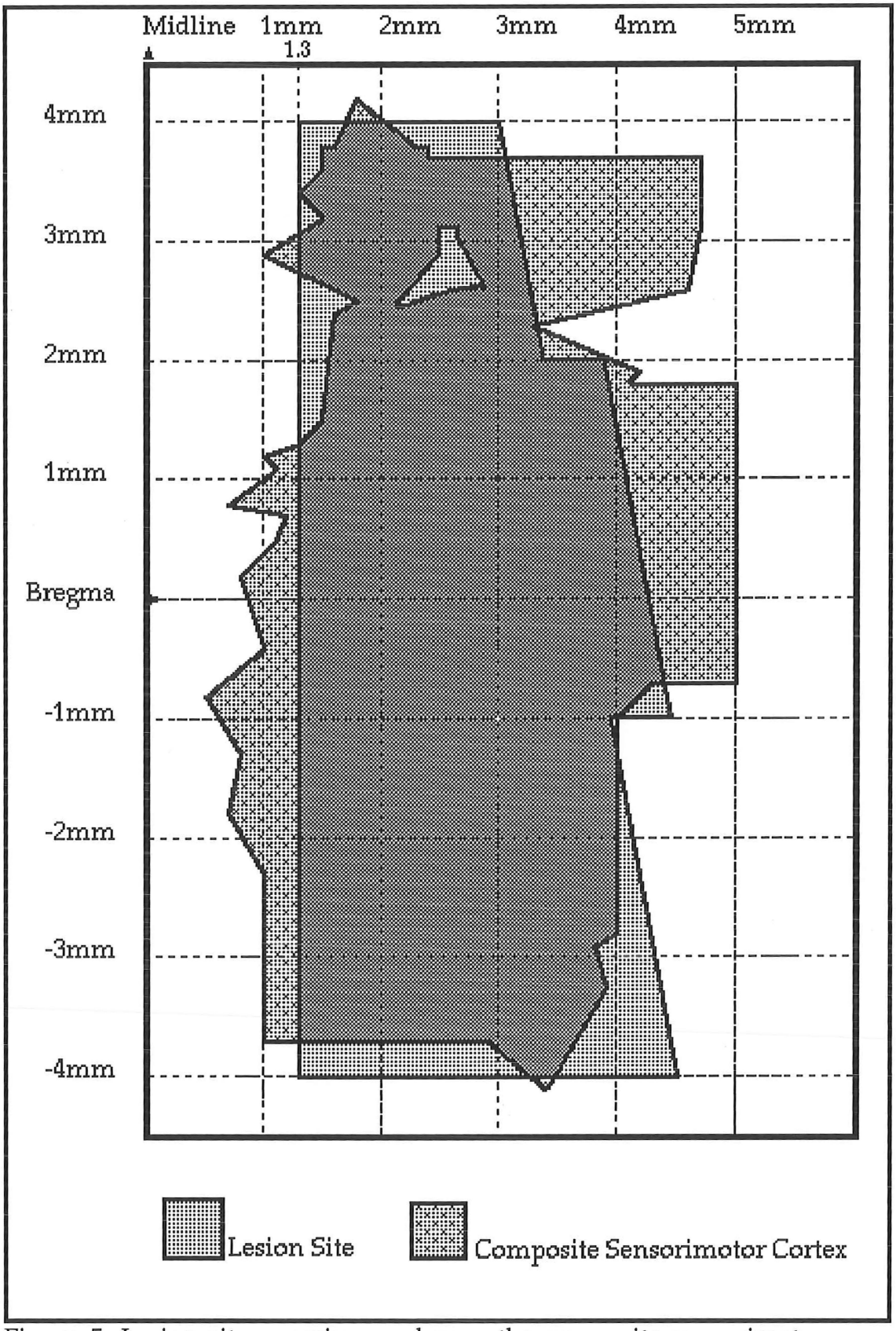

Figure 5. Lesion site superimposed over the composite sensorimotor cortex.

30 days after lesion surgery, the transplant groups received grafts of frontal cortex suspension tissue into an area just lateral of the midline in the cortex adjacent to the lesion (Figure 6). The animals were anaesthetised and the tissue was transplanted in 4 locations: 0.8mm lateral to the midline, 0.22mm ventral to the dorsal cortical surface and at -3mm and -1mm anterior to Bregma and 1mm and 3mm posterior to Bregma, in that order. Each 2µl of suspension was injected over a 2 minute period plus the syringe was left *in situ* for a further 3 minutes after injecting. Once this was completed the animal was removed, sutured, antibiotic cream applied to the wound and then placed in an individual cage with food and water available *ad libitum*.. All non-transplanted animals received scalp incision and suturing only.

2.3.2 CINEMATOGRAPHY

All animals were filmed while crossing the beam the day before lesion surgery. On each occasion, rats were filmed at least twice while crossing the entire length of the beam., film speed was 64 frames a second and the rats performed normally once they began the task. The rats were filmed twice after the transplant operations, firstly between the first and second session after transplants, and secondly immediately after behavioural testing had finished. These filming sessions followed the same procedure as the pre-lesion session. Except that the film speed was adjusted for each animal to ensure the requisite number of frames per limb motor cycle were filmed. Actual film speed varied from 32 to 48 to 64 frames per second, the appropriate speed being judged by the cameraman and author (In order to guarantee a minimum of 8 frames per motor cycle: from the time the heel first left the beam surface until it touched again).

2.3.3. CONDITION & ENVIRONMENTAL PLACEMENT.

The subjects were assigned into their experimental conditions shortly after lesion surgery. The process involved ranking the subjects behavioural performance in order to ensure that all groups were matched on all types of behaviour. The data used to rank the rats was drawn from the last 5 prelesion sessions, the data was averaged and the rats ranked according to speed and then consecutively divided into groups of 5. The first rat of the first group was assigned into the first condition, the second rat of the first group into the second condition etc, once the entire group had been placed the second group was assigned in reverse order, the third group in the original order and so on until all rats were assigned to an experimental condition. Subsequent statistical analysis revealed no significant differences between groups before lesion surgery (see below).

Subjects were placed back into their prelesion cages 7 days after lesion surgery and remained there until transplant surgery. Immediately following transplant surgery subjects were placed into individual cages (to allow for ease of postsurgery monitoring) with food and water available *ad libitum*, for 24 hours. After 24 hours they were placed into their appropriate housing environment. They remained in their appropriate housing environment continually thereafter, except that the enriched group were transferred into standard cages during testing (3-4 hours) and returned to the enriched environment once testing was completed for the day.

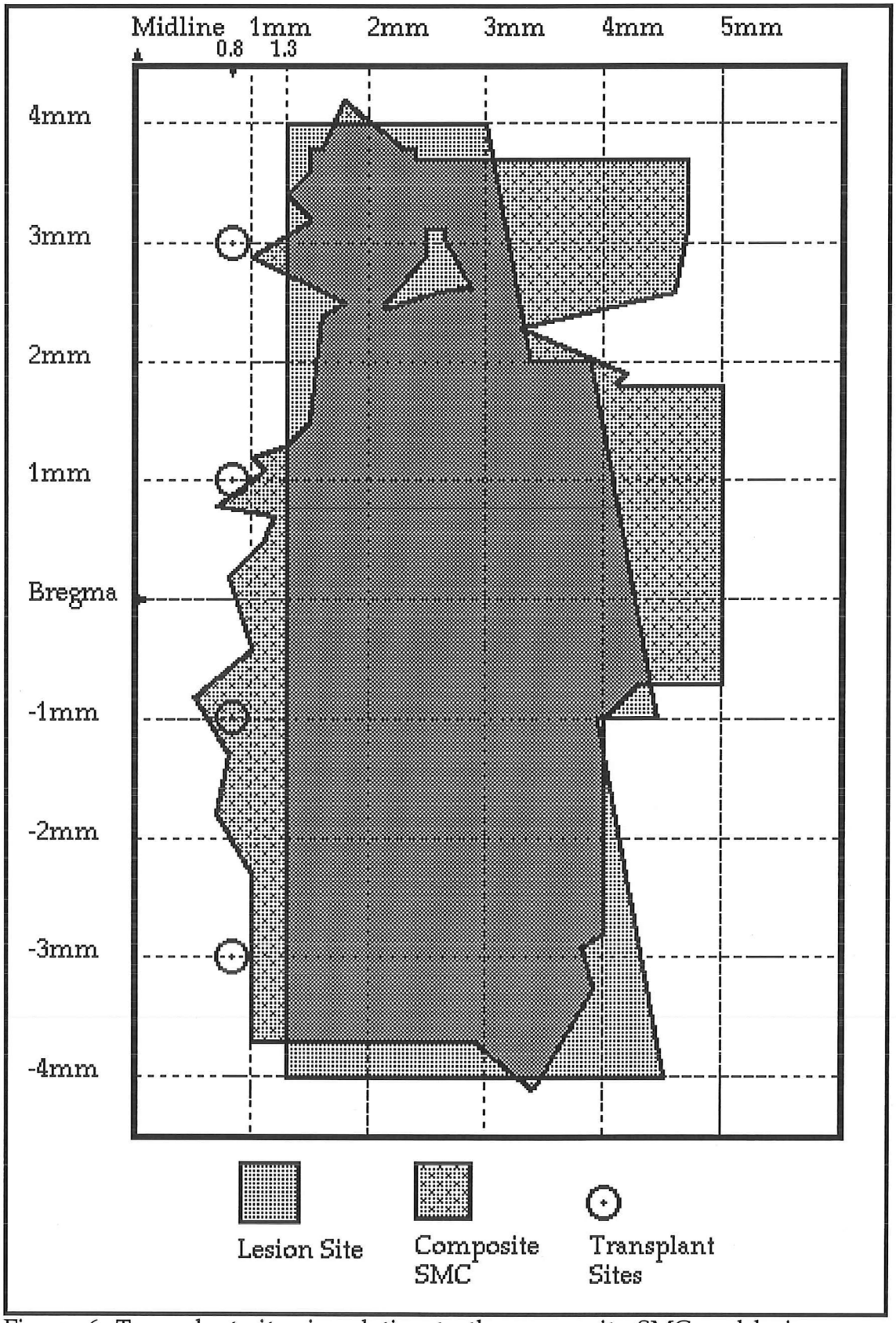

Figure 6. Transplant sites in relation to the composite SMC and lesion sites.

2.3.4 BEHAVIOURAL TRAINING AND TESTING.

BEHAVIOURAL TRAINING.

The rats were first given training sessions using the 5cm wide beam. In the first session each animal was allowed to explore the beam for 10 minutes. For the 2nd. 10 minute session the animals were encouraged (with chocolate placed along the length of the beam) to transverse the beam from the starting platform to the goal box. From the 3rd. session onwards, the animals were trained to traverse the 2.5cm. wide beam. For the 3rd and 4th sessions they animals each spent 10 minutes on the beam, performing the task with the food reward in the goal box only. From the 5th. session onwards the animals were allowed to make 10 crossings (trials) per session, regardless of the time taken. From the 5th. session onwards, behavioural measures were recorded. Training took place twice a week, with the first half of the animals being trained on the first one day, and the second half on the next. By the end of the training period (8 sessions) all the animals were able to perform the task quickly and virtually error free.

BEHAVIOURAL TESTING

At the half way point of the post-lesion/pre-transplant delay the subjects were tested on the beam task to ensure that the lesion groups were not significantly different on any measure. This was also done in order to ensure the lesions had not resulted in significantly different impairments. 5 trials were used instead of 10 in order to minimise the amount of task re-exposure the subjects experienced prior to testing for recovery after transplants and environmental placement. Statistical

analysis revealed no significant difference between any lesion group on any behavioural measurement (see below). Lesion animals were significantly impaired to controls for all behaviours (see below).

All enriched animals spent 7 days in the enriched environment before they were tested, and they remained within the environment throughout the test period of 12 sessions (7 weeks). Testing followed exactly the procedure used for the last 5 pre-surgery training sessions except that the order in which animals were tested was now half the enriched and half the standard animals, in a random order, on one day and the remaining animals the next day. This order was strictly adhered to for all subsequent sessions. Daily, after each session, the rats were fed a restricted diet to maintain 85% of *ad libitum* body weight.

2.3.5 BEHAVIOURAL MEASUREMENT.

BEAM RUNNING. Two separate measures were recorded: 'run time', the time taken to cross the beam, and 'foot errors'. An animal made a foot error if the distal tip of the middle digit on the hind limb crossed one of the foot error lines drawn on the beam. The top line was labelled "type 1", the bottom line "type 4" . The error types were exclusive with the 'worst' error type being record (ie. a type 3 error would not also be recorded as a type 1 and type 2 error). At the end of each session an average score was computed for all 6 measurements (each foot error type, error frequency and the reciprocal of the run time mean).

From the behavioural data 7 dependent variables were analysed,

1) Reciprocal run time. The reciprocal run-time of the average of each session, for each animal (To account for variance between session variances, see below).
2) Error frequency for error types 1-4 . The average number of each error type each rat made per session.
3) Weighted-error-total, which was derived by weighting the appropriate error types according to their degree. The average of type 1 errors were multiplied by 1, the average of type 2 errors multiplied by 2 etc and the results added together to produce a single figure for each rat for each session.
4) Error frequency, the number of errors made, regardless of type.

The raw run-time scores were transformed to the reciprocal to produce homogeneity of variance across sessions. Similarly the initial postransplant session's variance was far higher than that of the later sessions (for all dependent variables) so these too were analysed independently of the later sessions (2-12), as per Held et al (1985).

CINEMATOGRAPHIC ANALYSIS. Analysis of the film data was carried out as per Held et al (1985). Each trajectory curve was normalised to adjust for differences in amplitude and extent in order to permit comparisons of movement topology. The normalisation involved setting the maximum amplitude of each curve equal to one and adjusting the vertical displacement of all other points on the curve as a proportion of

the maximum amplitude. Likewise, the maximum forward (horizontal) displacement was equated to one, and the horizontal value of all other points was expressed as a proportion of the maximum displacement. Then the curves were analysed for their areas and an average area calculated. Three normalised hind paw curves were analysed for each animal to generate the area under the curve. Only one normalised front paw curve was analysed, in a like manner to the hind paw analysis, for each animal due to time constraints. The trajectory for both limbs was measured from the co-ordinates for the distal tip (ie. the tip of the third digit) of the appropriate paw.

2.3.6 HISTOLOGY

At the conclusion of the experiment, 8 weeks after grafting, the rats were sacrificed with a Nembutal overdose and perfused with saline and 4% formalin. After 48 hours in 4% formalin at 4°C the brains were transferred into a long term solution of sucrose formalin and stored at 4°C. The brains were then cut in a cryostat at 50μm, and 2 adjacent frozen sections, of every 12, were stained and mounted, one for AChE and the other for cresyl violet. Examination of the brains later resulted in seven subjects being removed from the analysis due to five cases of excessively large lesions, one case of a nonsurviving transplant and one case of gross hydrocephaly in the ipsilateral hemisphere. The resulting experimental group are shown in table 1.

2.3.7 CALCULATION OF LESION & TRANSPLANT VOLUMES.

A Genius Hisketch tablet was used to trace the surface area of the lesions and transplants from all mounted sections. The volume of the

lesions and transplants were calculated using the following formula (from Dunnett et al, 1986):

$$V = \sum A \times T \times F$$

where V is the volume (millimetres cubed), $\sum A$ is the sum of the areas of the individually traced outlines (in millimetres squared, previously corrected for magnification), T is the section thickness (50μ) and F is the frequency of sections traced (1:6).

Table 1 Experimental Group Numbers.

Experimental Condition.	Group Size.
Control.	11
Nontransplant-Enriched.	7
Nontransplant-Standard.	8
Transplant-Enriched	6
Transplant-Standard.	9
Total.	41

3.0 Results

3.1 HISTOLOGY

LESIONS

On the basis of the neurological studies consulted (Hall and Lindholm 1974; Hicks and D'Amato 1975; Neafsey et al 1982, Neafsey et al 1986 and Zilles, 1985) all lesioned group were considered to have effective removal of the right SMC. Visual analysis showed that the lesions imposed in this study completely encompassed the motor representation areas of the front and hind paws, and the primary motor cortex (for example Figure 7). The anterior-posterior and medial-lateral extent of the aspiration were relatively consistent but the ventral extent of the lesions were more variable, in all cases reaching the dorsal surface of the hippocampus, but in some cases also slightly invading the hippocampus. In 5 cases invasion caused unacceptable damage and the subjects were removed from the analysis. Analysis of variance (2 x 2, Transplant x Environment) of the lesion volume (Figure 8, left panel) showed no significant differences between groups ($F<1.0$, $P>0.1$). Correlation's between lesion volume and run time, and weighted-error-total revealed that there was no significant correlation between lesion volume and those behaviours ($r=0.2$ & 0.233, respectively, $P>0.1$).

TRANSPLANTS

Of the 15 rats that received transplants, 14 were found to have surviving grafts (example Figure 9). Apart from survival, position, general

viability and volume, no other transplant measurements was performed. A t-test revealed no significant differences in transplant volumes between the transplant-enriched and transplant-standard groups (Figure 8, right panel, t=0.67 with 13 df, P>0.1). Correlation's between transplant volume and run time, and weighted-error-total revealed that there was no significant correlation between transplant volume and those behaviours (r=0.24 & 0.18, respectively, P>0.1).

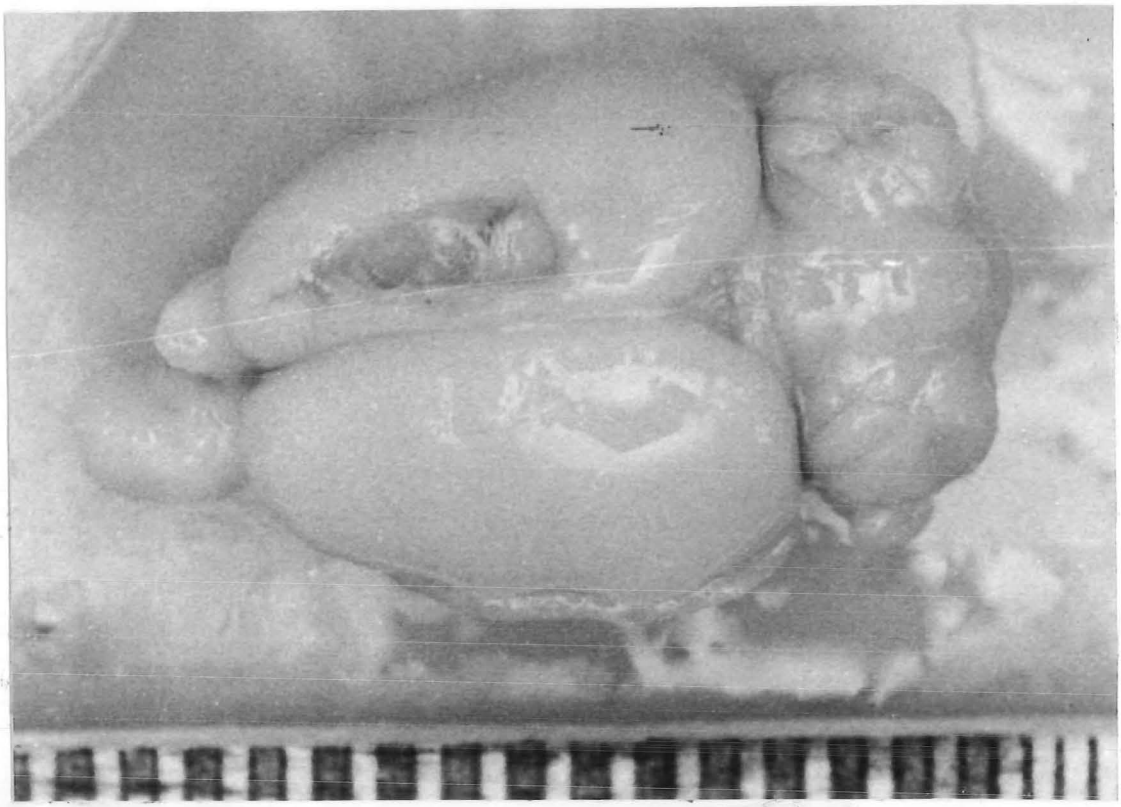

Figure. 7, Photograph of a Representative Lesion.

3.2 BEHAVIOURAL ANALYSIS

3.2A PREOPERATIVE PERFORMANCE

Analyses of variance (2 x 2 x 4, Transplant x Environment x Session, with repeated measures on the third factor) was performed on the last 5 sessions pre-lesion-surgery, for both run time and weighted-error-total (Figure 10, left and right panel respectively). At this time there were no differences between any lesion

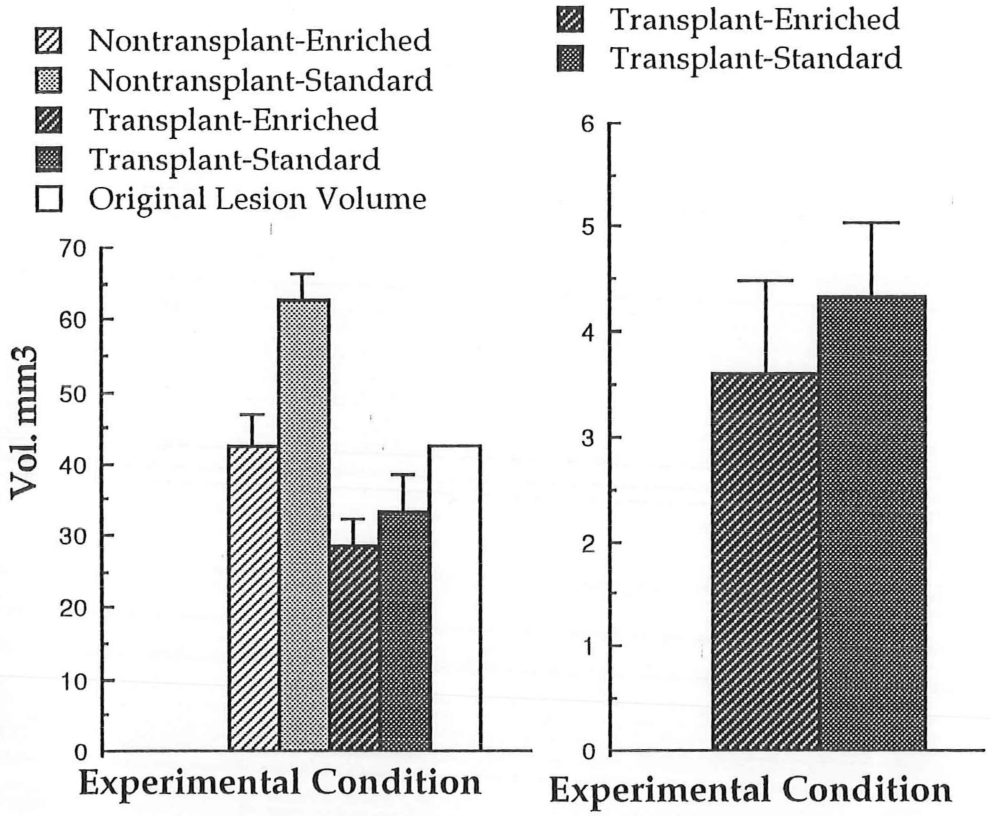

Figure 8. Lesion and Transplant volumes (left and right panel respectively), as determined by low magnification tracing of sections, averaged across groups. Vertical bars represent the S.E.M.

groups on either measure (Run Time: Transplant ($F<1.0$,$P>0.1$), Environment ($F<1.0$,$P>0.1$) and Transplant x Environment ($F<1.0$,$P>0.1$). Weighted-error-total: Transplant ($F<1.0$,$P>0.1$), Environment

(F(1,33)=2.51, P>0.1) and Transplant x Environment (F<1.0 ,P>0.1). As well t-tests were calculated to compare each lesion group, individually, against the control group. Again there were no significant differences for either measure (All lesion groups independently compared to controls. Run time: nontransplant-enriched,t=0.22 with df 16, P>0.1 nontransplant-standard, t=0.547 with df 17, P>0.1 transplant-enriched, t=0.8 with df 15, P>0.1 and transplant-standard, t=1.04 with df 18, P>0.1. Weighted-error-total: controls compared to nontransplant-enriched, t=0.8 with df 16, P>0.1 nontransplant-standard, t=0.54with df 17, P>0.1 transplant-enriched, t=0.22 with df 15, P>0.1 transplant-standard, t=1.04 with df 18, P>0.1.Therefore there was behavioural parity between all groups before lesion surgery.

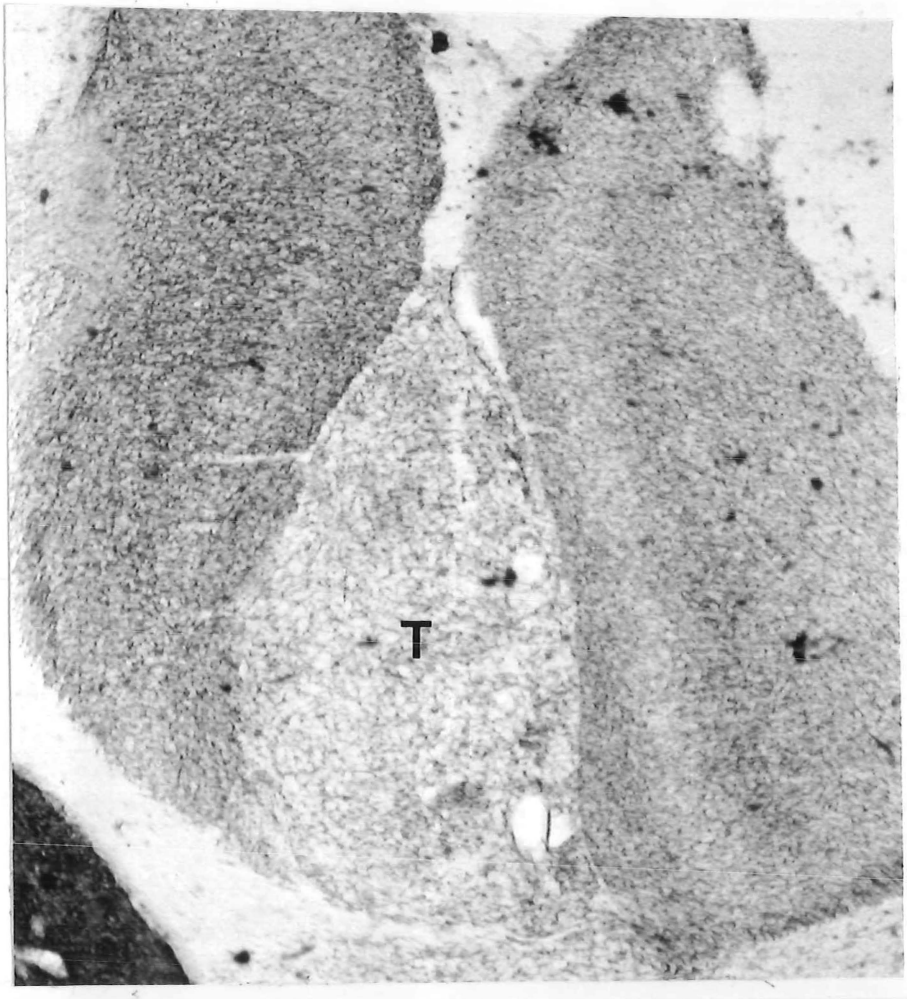

Figure. 9, Photograph of a Frontal Cortex Transplant (T) in the SMC.

Analysis of the hind paw area-under-the-curve was performed to compare the areas of all the groups at the pre-surgery film session, both between individual lesion groups and controls (t-test), and between the lesion groups (2 x 2 ANOVA, Transplant x Environment). No significant differences were found between controls and lesion groups, except that control and nontransplanted-enriched group test approached significance. Controls compared to: nontransplant-enriched, $t=2.12$ with 16 df, $P=0.05$ nontransplant-standard, $t=0.67$ with 17 df, $P>0.1$ transplant-enriched, $t=0.32$ with 15 df, $P>0.1$ and transplant-standard, $t=0.46$ with 18 df, $P>0.1$). The ANOVA revealed no significant differences for both main effects and interactions (Transplant ($F(1,26)=3.64$, $P>0.05$), Environment ($F(1,26)=2.15$, $P.>0.1$) and Transplant x Environment ($F<1.0$, $P>0.1$).

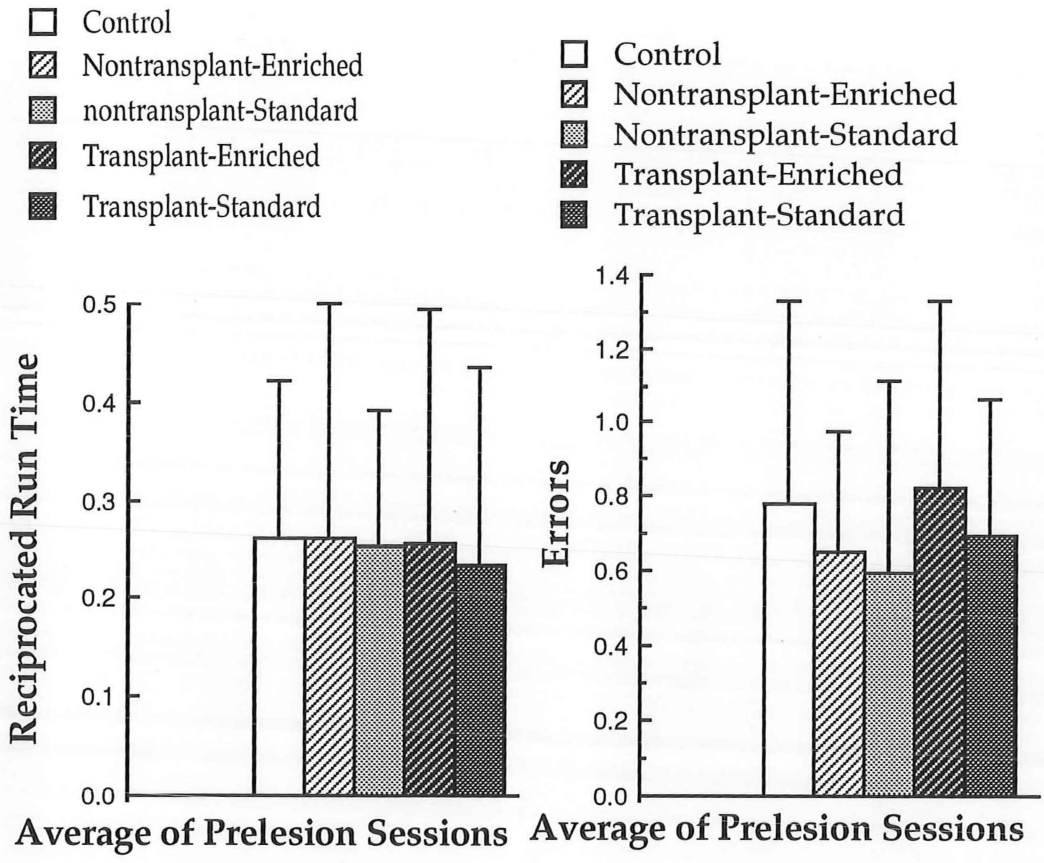

Figure 10. Preoperative locomotor performance for run time and weighted-error-total (left and right panel respectively) averaged over the last 4 sessions before lesion surgery. Vertical bars represent the S.E.M.

3.2B. POSTLESION/PRETRANSPLANT PERFORMANCE.

Analyses of variance (2 x 2, Transplant x Environment) was performed on the postlesion-pretransplant data, for all behavioural measures (Figure 11, reciprocal run time and weighted-error-total, left and right panel respectively). At this time there were no differences between any lesion groups on any measure, Run time: F's<1.0, P>0.1; Error type 1: F's<1.0, P>0.1; Error type 2: F(1,29)=1.638, P>0.1; Error type 3: F's<1.0, P>0.1 and Error type 4: F's<1.0, P>0.1, Weighted-error-total: F's<1.0, P>0.1 (all F values are drawn from the result of the Transplant x Environment interaction). T-tests were performed to examine any differences between lesion groups and the control group. All lesion animals were significantly

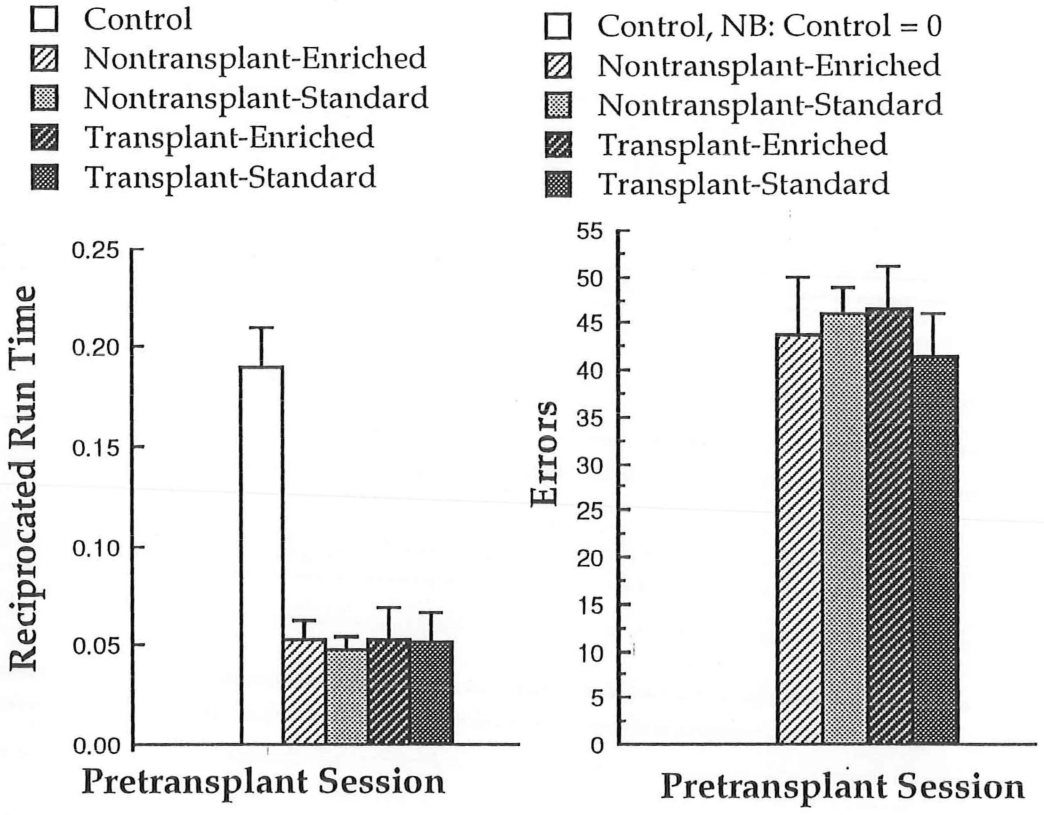

Figure 11. Postlesion/pretransplant locomotor performance for run time and weighted-error-total (left and right panel respectively) as averaged across a partial (5 trial) session. Vertical bars represent the S.E.M.

slower, and made significantly more errors, than controls (All lesion groups compared to controls: Run time, t's >4.68, P<0.01). Error type 1, t's >2.31, P<0.025; Error type 2, t's >3.81; P<0.025; Error type 3, t's >2.19, P<0.025 and Error type 4, t's >7.09, P<0.01, with df 16 (nontransplant-enriched), 17 (nontransplant-standard), 15 (transplant-enriched) and 18 (transplant-standard). Due to very low control group scores the Mann Whitney U test was used to compare controls and lesion groups for the weighted-total-error. All lesion animals made more weighted-error scores than controls, Z's>3.663, P>0.1. Therefore while lesion surgery produced a significant behavioural impairment the lesion groups remained behaviourally equivalent. Thus any behavioural differences between lesion groups thereafter were not a result of lesion variation.

3.2C POSTRANSPLANT PERFORMANCE

Analysis was performed upon each of the 7 dependent variables. 1) Reciprocal run time, 2)-5) Errors types 1-4, respectively, 6) Weighted-error-total, and 7) Error frequency. Data analysis was performed in a manner similar to Held et al (1985) in that the analysis was divided into two sections *initial postransplant performance*, being the first session of testing after transplanting, and *subsequent postransplant performance*, being the remaining sessions of the testing period (Sessions 2 to 12). This division was performed as there was a very high degree of variability in the data at the initial session, in comparison to the subsequent sessions.

For both the initial postransplant session and the subsequent sessions two sets of analyses were performed. First each of the lesion group were individually compared to the control group to determine if

the two groups were significantly different or not. During analysis of the initial session this took the form of a t-test. During analysis of the subsequent sessions an ANOVA was employed, where the design was 2 x 11 (Control/Lesion status x Session, with repeated measures on the last factor). Secondly the lesion groups were compared to determine any significant differences. During analysis of both the initial and subsequent postransplant sessions the analysis took the form of an ANOVA. The ANOVA design for the initial session was 2 x 2 (Transplant x Environment). Design of the ANOVA for the subsequent sessions analysis was 2 x 2 x 11 (Transplant x Environment x Session, with repeated measures on the last factor).

3.2.1 RECIPROCAL RUN TIME

Initial Postransplant Performance: At the initial postransplant session all lesion groups were significantly slower than controls (Figure 12, left panel). Controls compared to: nontransplant-enriched, $t=3.36$ with 16 df, nontransplant-standard, $t=3.35$ with 17 df, transplant-enriched, $t=1.757$ with 15 df and transplant-standard $t=2.61$ with 18 df, $P<0.001$ for all tests. However the transplanted-enriched group came extremely close to being not significantly slower than controls ($P=0.497$). The ANOVA revealed that there was no significant difference for running time between any of the lesion groups (Transplant $F(1,26)=2.27, P>0.1$). Both the main Environment effect and the Transplant x Environment interaction ($F<1.0$, $P>0.1$).

Subsequent Postransplant Performance: There was no significant difference in running time between any of the lesion groups when compared to the control group, throughout the subsequent sessions

(Controls compared to: nontransplant-enriched ($F<1.0$, $P>0.1$), nontransplant-standard ($F(1,170)=3.58$, $P>0.05$), transplant enriched ($F<1.0$, $P>0.1$) and transplant-standard ($F<1.0$, $P>0.1$) even though the graph (Figure 12, right panel) suggests that the nontransplant-standard group was slower than the control group. None of the Condition x Session interactions were significant (nontransplant-enriched ($F<1.0$, $P>0.1$), nontransplant-standard ($F<1.0$, $P>0.1$, transplant enriched ($F<1.0$, $P>0.1$) and transplant-standard ($F(10,180)=1.02$, $P>0.1$).

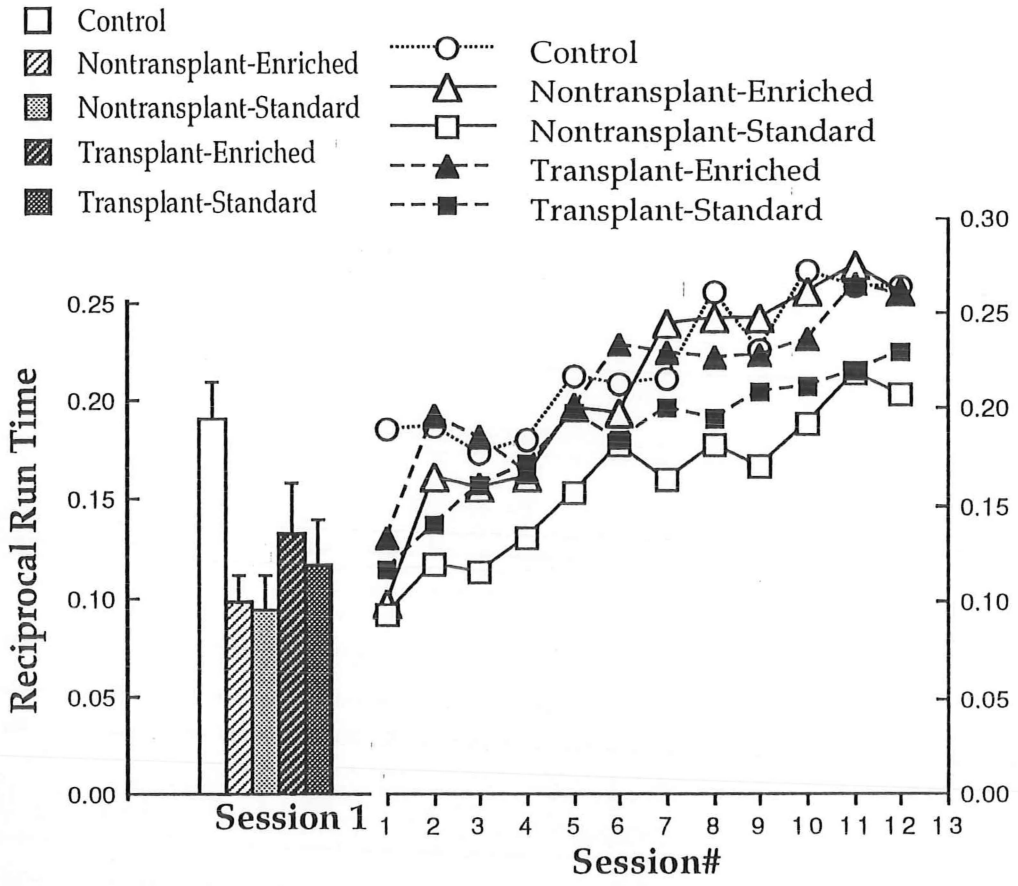

Figure 12. Reciprocal Run Time, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

The presence or absence of a transplant or enriched environment, individually or in interaction, did not alter recovery, as all lesion groups did not have significantly different run times, Transplant

($F<1.0, P>0.1$), Environment ($F(1,26)=1.67$, $P>0.1$), Transplant x Environment $F<1.0, P>0.1$), Transplant x Session $F(10,260)=1.02, P>0.1$), Environment x Session $F(10,260)=1.04, P>0.1$) and Transplant x Environment x Session ($F(10,260)=1.10$, $P>0.1$). However all groups, including controls, experienced a significant increase in speed (reduction in time across the beam) during sessions two to twelve ($F(1,260)=7.14$, $P<0.001$ and $F(10,260)=25.021$, $P<0.01$ respectively). While the control groups did not differ between the prelesion session and the initial postransplant session, the lesion groups all experienced significant impairment as a result of lesioning (postlesion-pretransplant and initial postransplant sessions analysis) but all lesion groups improved between the postlesion/pretransplant session and the initial postransplant session (Figures 10 and 12, respectively). The lesion groups also experienced total recovery in so far as they reached parity with controls, at session 2 and were not different thereafter.

3.2.2 Error Type One

Initial Postransplant Performance: The lesion groups made significantly more type one errors than the control group (Figure 13, left panel). Controls compared to: nontransplant-enriched $t=13.63$ with 16 df, nontransplant-standard $t=8.04$ with 17 df, transplant enriched $t=5.25$ with 15 df and transplant-standard $t=17.34$ with 18 df, $P<0.001$ for all tests. During the initial postransplant test session the enriched group made significantly more type one errors than the standard group ($F(1,26)=4.27$, $P<0.025$). Examination of Figure 13, left panel, reveals that this was very likely due to the high level of error performance made by the nontransplanted-enriched group. The Transplant effect approached significance ($F(1,26)=2.92, 0.1<P>0.05$) again most likely due to the poor

performance of the nontransplanted-enriched group, however there was no significant Transplant x Environment interaction (F(1,26)=2.55,P>0.1).

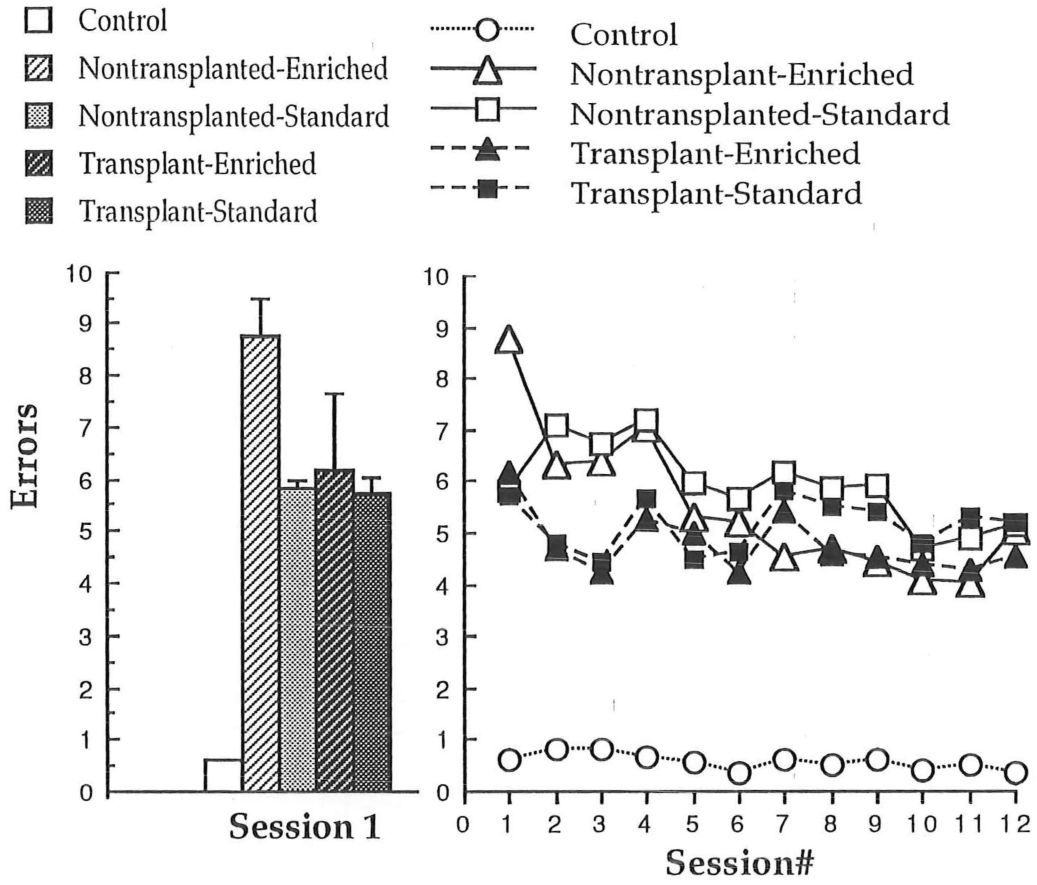

Figure 13. Error Type 1, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

Subsequent Postransplant Performance: All lesion groups made significantly more type one errors than the control group, over sessions. Controls compared to: nontransplant-enriched (F(1,160)=73.84 P<0.001), nontransplant-standard (F(1,170)=66.81, P<0.001), transplant enriched (F(1,150)=93.41, P<0.001) and transplant-standard (F(1,180)=104.04, P<0.001). The nontransplanted groups versus control group Condition x Session interactions were significant, nontransplant-enriched (F(10,160)=5.12, P<0.001) and nontransplant-standard (F(10,170)=4.14, P<0.001) while the transplanted groups versus control group Condition x

Session interactions were not, transplant enriched, ($F<1.0$, $P>0.1$) and transplant-standard ($F(10,180)=1.64$, $P>0.05$). Interestingly, while the enriched groups did not make more errors after session one than the other lesion groups, they also did not make significantly less of this error type as they did for error types 3,4, and weighted-error-total.

Analysis revealed that, as a whole, the lesion groups experienced a significant decrease in error performance over sessions ($F(10,260)=4.88$, $P<0.01$). There was also a Transplant x Session interaction ($F(10,260)=4.053$, $P<0.001$) for sessions two to twelve. Examination of the simple effects within this interaction revealed that during postransplant test sessions two, and three the transplanted group made less errors than the nontransplanted groups (Session 2: $F(1,260)=6.63$, $P<0.025$, session 3: $F(1,260)=8.37$, $P<0.01$). However after session 4 there was no difference between the transplant groups ($F<1.0$,$P>0.1$). While the nontransplanted group showed a significant reduction in error performance over sessions ($F(10.,260)=7.63$,$P\leq0.01$) the transplanted and control groups did not ($F(10,260)=1.5$, $P>0.1$, and $F<1.0$,$P>0.1$, respectively).

There was no main effect of either transplant or environment ($F(1,26)=1.21$, $P>0.1$ and $F<1.0$, $P>0,1$, respectively). Similarly there was no Environment x Session, nor Transplant x Environment x Session, interactions ($F<1.0$, $P>0.1$ and $F<1.0$, $P>0.1$, respectively).

3.2.3 Error Type Two

Initial Postransplant Performance: Lesion groups made significantly more type two errors than the control group (Figure 14, left panel). Controls compared to: nontransplant-enriched $t=4.92$ with 16 df,

nontransplant-standard t=3.16 with 17 df, transplant enriched t=2.35 with 15 df and transplant-standard t=5.73 with 18 df, P<0.001 for all tests. Figure 14, left panel, might suggest that the enriched groups made less errors than the standard groups at the initial session, but this difference only approached significance (F(1,26)=3.36, P>0.05). Furthermore there was no Transplant effect (F<1.0, P>0.1) nor was there a Transplant x Environment interaction (F<1.0,P>0.1).

Subsequent Postransplant Performance: All lesion groups made significantly more type two errors than the control group, over sessions. Controls compared to: nontransplant-enriched (F(1,160)=19.75, P<0.001),

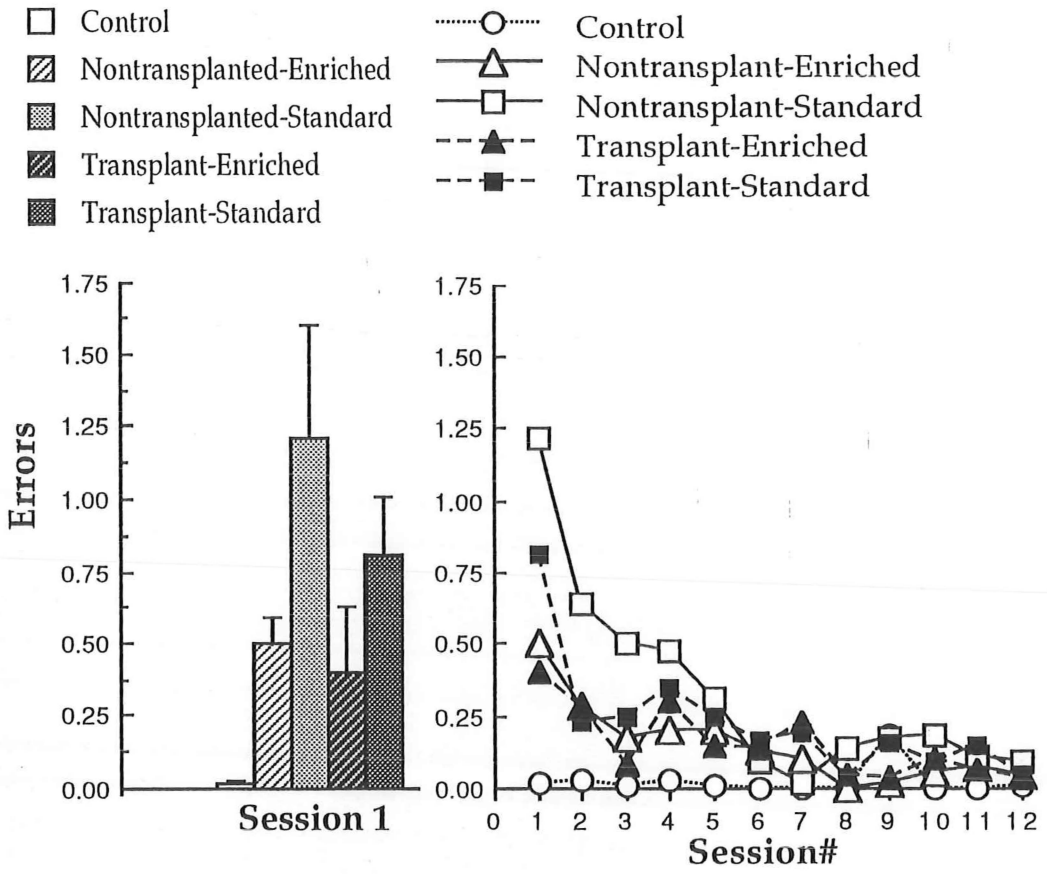

Figure 14. Error Type 2, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

nontransplant-standard ($F(1,170)=15.98$, $P<0.001$), transplant enriched ($F(1,150)=18.27$, $P<0.00$ and transplant-standard ($F(1,180)=20.95$, $P<0.001$). Furthermore all the Condition x Session interactions were significant, nontransplant-enriched ($F(10,160)=2.15$, $P<0.025$), nontransplant-standard ($F(10,170)=4.91$, $P<0.001$), transplant enriched ($F(10,150)=4.7$, $P<0.001$) and transplant-standard ($F(10,180)=3.30$, $P<0.001$). The only significant result produced during the lesion group analysis was that the lesion groups as a whole experienced a significant decrease in error performance over sessions ($F(10,260)= 8.45$, $P<0.01$). Otherwise there was no difference in error performance between the transplant groups for the subsequent sessions ($F<1.0, P>0.1$) nor were any of the transplant interactions significant (Transplant x Environment ($F<1.0, P>0.1$), Transplant x Session ($F(10,260)=1.26$, $P>0.1$) and Transplant x Environment x Session ($F<1.0$, $P>0.1$). The Environment x Session interaction was not significant, ($F(10,260)=1.07$, $P>0.1$) and the main environment effect only approached significance ($F(1,26)=3.05$, $P>0.05$). The control group performed virtually no type 2 errors and their type 2 error performance did not alter across sessions ($F(10,260)=4.887$, $P>0.1$).

3.2.4 Error Type Three

Initial Postransplant Performance: Unlike the previous measures, the nontransplanted-enriched groups did <u>not</u> make significantly more type three errors than the control group ($t=1.24$ with df 16, $P>0.1$) (Figure 15, left panel). The other lesion groups made significantly more type three errors than the control group. Controls compared to: nontransplant-standard $t=4.03$ with df 17, transplant enriched $t=2.71$ with df 15 and transplant-standard $t=4.12$ with 18 df, $P<0.01$ for all tests. Examination of the lesion group performances in

Figure 15, left panel, clearly shows that the enriched groups made significantly less errors than the standard groups, and the statistical analysis bears that out ($F(1,26)=11.98$, $P<0.005$). There were no other significant differences between lesion groups during the initial session, Transplant ($F<1.0$, $P>0.1$) and Transplant x Environment ($F<1.0$, $P>0.1$).

Subsequent Postransplant Performance: All lesion groups made significantly more type three errors than the control group, over sessions. Controls compared to: nontransplant-enriched ($F(1,160)=13.90$, $P<0.001$), nontransplant-standard ($F(1,170)=18.65$), $P<0.001$, transplant enriched ($F(1,150)=23.82$, $P<0.001$) and transplant-standard ($F(1,180)=23.32$, $P<0.001$). However there were not any significant Condition x Session interactions: nontransplant-enriched ($F(10,160)=1.35$, $P>0.1$), nontransplant-standard ($F(10,170)=1.78$, $P>0.05$), transplant enriched ($F(10,150)=1.10$, $P>0.1$) and transplant-standard ($F(10,180)=1.46$, $P>0.1$). The ANOVA between lesion groups reveals that the enriched group, as a whole, made significantly less type 3 errors than the standard group ($F(1,26)=10.85$, $P<0.005$). There were no other significant effects or interactions within the lesion group analysis (Transplant ($F<1.0$, $P>01$), Transplant x Environment ($F(1,26)=1.51$, $P>0.1$ Transplant x Session ($F<1.0$, $P>0.1$), Environment x Session ($F(10,260)=1.06$, $P>0.1$) and Transplant x Environment x Session ($F(10,260)=1.15$, $P>0.1$). Unlike other error types, there was <u>not</u> a significant session effect for the lesion animals as a whole ($F(10,260)=1.15$, $P>0.1$). This is consistent with Figure 15, right panel, in which only the nontransplanted and transplanted-standard groups show any sign of downward movement over sessions, and even that movement was uncertain and, especially so for the transplanted-standard group.

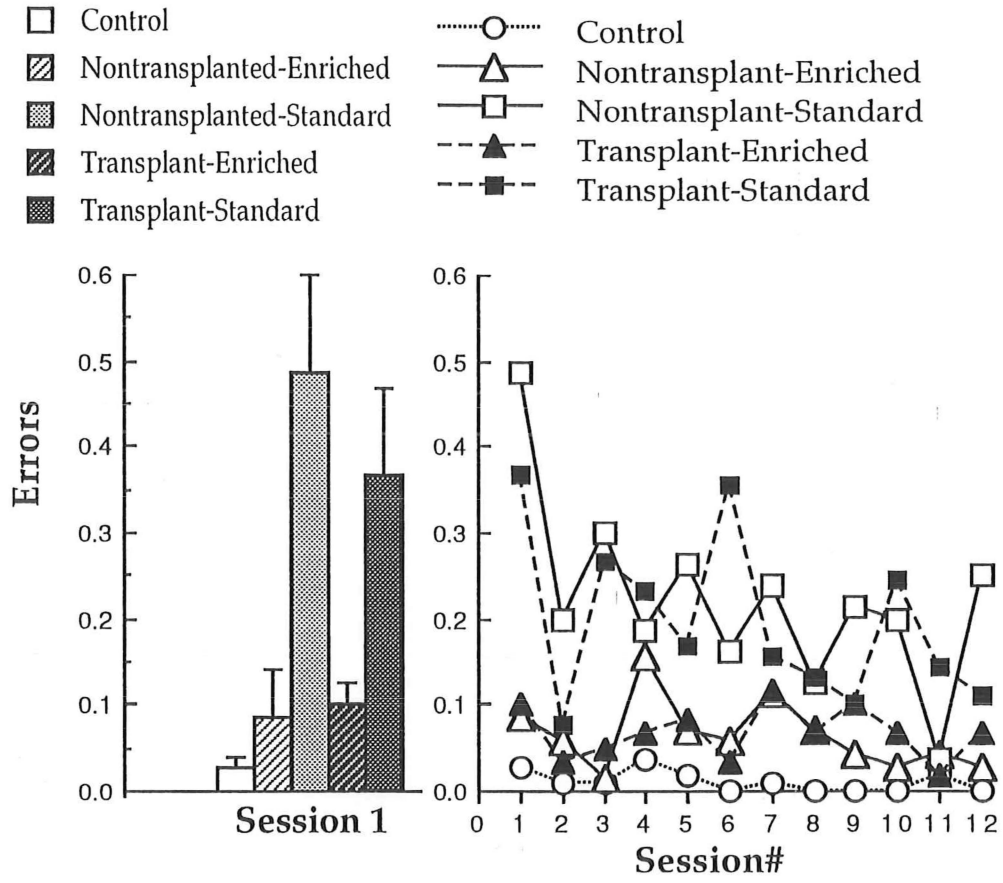

Figure 15. Error Type 3, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

3.2.5 Error Type Four

Initial Postransplant Performance: Due to a lack of variation in the control group scores a Mann-Whitney U test, instead of a t-test, was used to compare control and lesion groups. Lesion groups made significantly more type four errors than the control group (Figure 16, left panel). Controls compared to: nontransplant-enriched $Z=2.22$, nontransplant-standard $Z=3.31$, transplant enriched $Z=2.49$, transplant-standard $Z=3.34$, $P<0.001$ for all tests. As is obvious from the graph the enriched groups make significantly less type four errors than the standard groups ($F(1,26)=10.15$, $P<0.005$). However there was no significant difference between the transplant groups ($F(1,26)=2.87$, $P>1.0$), nor was

there a significant Transplant x Environment interaction (F(1,26)=2.46, P>0.1).

Subsequent Postransplant Performance: Statistics reveal that all lesion groups made significantly more type four errors than the control group, over sessions (Figure 16, right panel). Controls compared to: nontransplant-enriched (F(1,160)=13.90, P<0.001), nontransplant-standard (F(1,170)=18.65, P<0.001), transplant enriched (F(1,150)=23.82: P<0.001) and transplant-standard (F(1,180)=23.32, P<0.001). However there were not any significant Condition x Session interactions: nontransplant-enriched (F(10,160)=1.35, P>0.1), nontransplant-standard (F(10,170)=1.78),

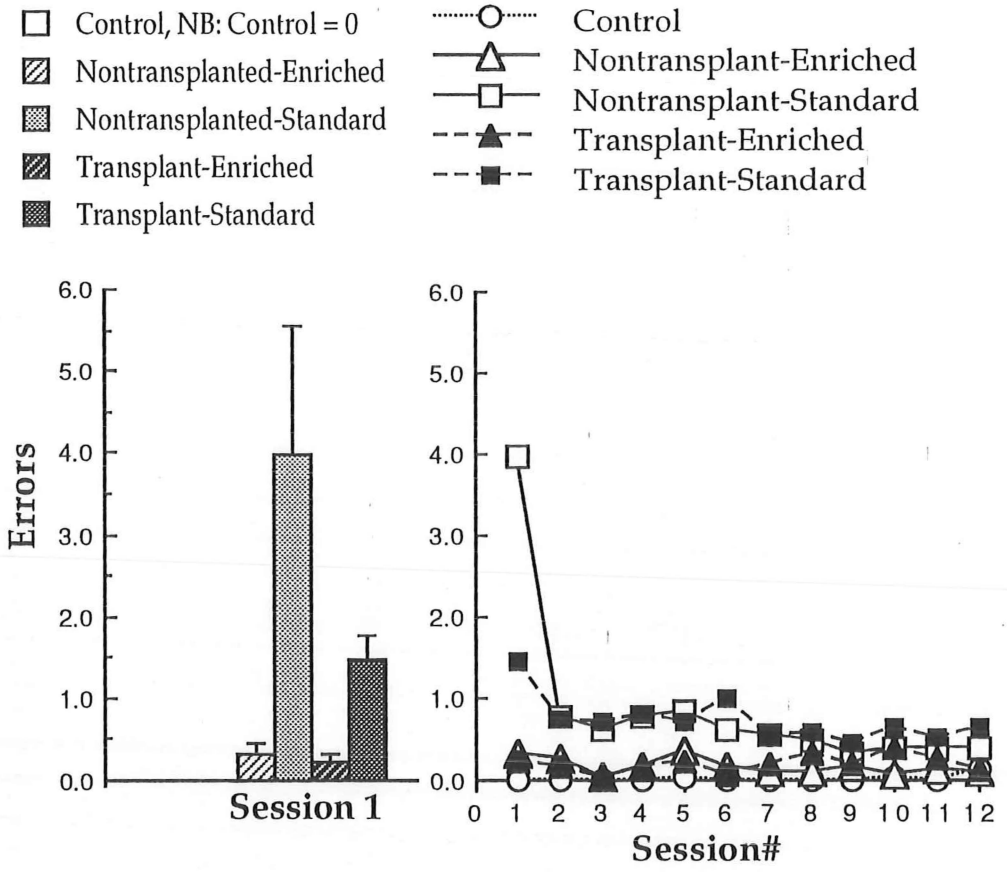

Figure 16. Error Type 4, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

P>0.05), transplant enriched (F(10,150)=1.10, P>0.1) and transplant-standard (F(10,180)=1.46, P>0.1).

As a whole the enriched groups made significantly less type four errors than the standard groups (F(1,26)=15.59, P<0.001). Moreover there was a significant Environment x Session interaction (F(10,260)=2.32, P<0.025) analysis of the simple main effects within this interaction revealed the enriched animals made significantly less errors than the standard animals at sessions 2-8 and 12. An examination of Figure 16, right panel, might suggest that there would be no session effect for the lesion groups as a whole, as the data paths after session 1 are fairly horizontal, however this was not confirmed by the statistics, rather the lesion groups as a whole did make less type four errors over sessions 2-12 (Albeit the significance was not as robust as for error types one and two) (F(10,260)=2.15, P<0.025). There were no other significant results as a result of the lesion group analysis (Transplant (F<1.0, P>0.1), Transplant x Environment (F<1.0, P>01), Transplant x Session (F(10,260)=1.13, P>0.1) and Transplant x Environment x Session (F<1.0, P.0.1).

3.2.6 WEIGHTED ERROR TOTAL

Initial Postransplant Performance: As may be expected from the prior analysis of the independent error types the lesion groups had a greater weighted-error score than the control group (Figure 17, left panel). Controls compared to: nontransplant-enriched t=5.06 with 16 df, nontransplant-standard t=26.4 with 17 df, transplant enriched t=5.07 with 15 df and transplant-standard t=11.35 with 18 df, P<0.001 for all tests. As both the previous analysis and the graph (Figure 17) predict, the enriched

groups had significantly lower weighted-error score than the standard groups (F(1,26)=8.82: P<0.01). What was not

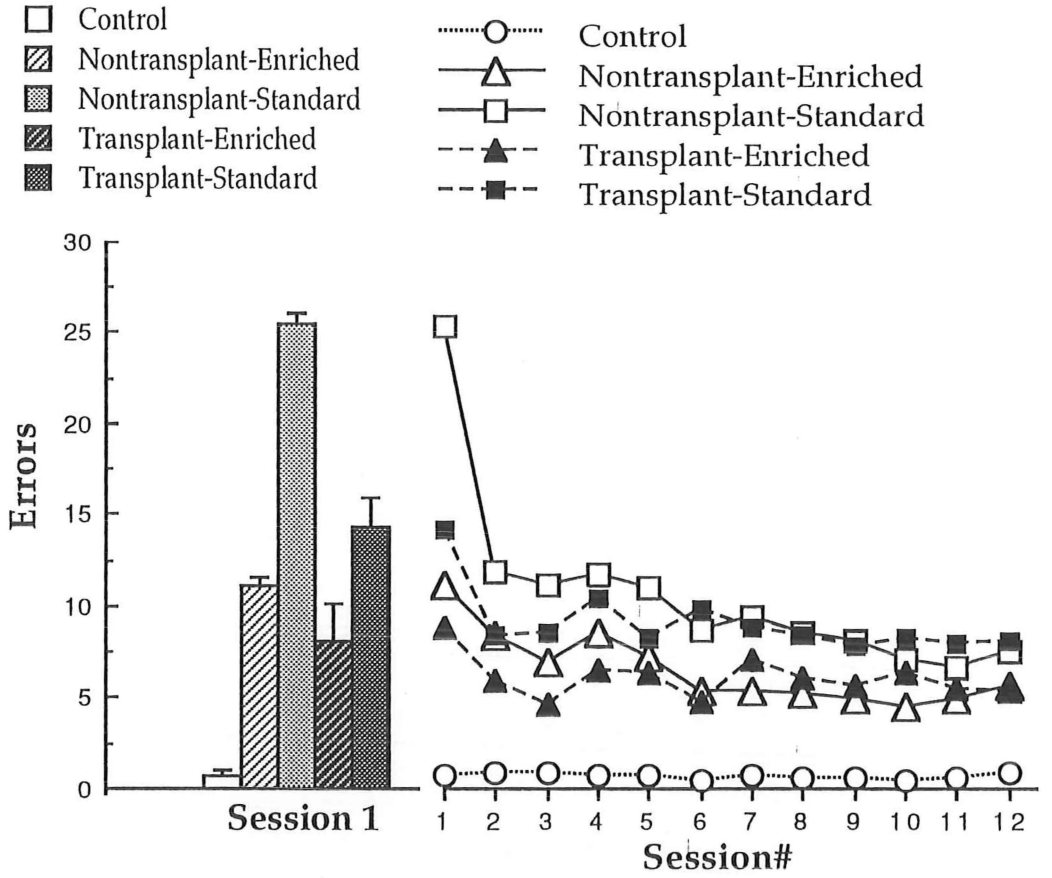

Figure 17. Weighted Error Total, the sum of all discrete error types multiplied by their degree, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

predicted by the previous analysis, but is by the graph, is that the transplanted groups had weighted-errors scores that were significantly lower than the nontransplanted groups (F(1,26)=4.23, P<0.05). However there was not a significant Transplant x Environment interaction (F(1,26)=1.26, P>0.1).

Subsequent Postransplant Performance: All lesion groups had significantly higher weighted-error scores than the control group, over

sessions (Figure 17, right panel) Controls compared to: nontransplant-enriched ($F(1,160)=60.38$, $P<0.001$), nontransplant-standard ($F(1,170)=46.24$, $P<0.001$), transplant enriched ($F(1,150)=71.54$, $P<0.001$) and transplant-standard ($F(1,180)=92.69$, $P<0.001$). Furthermore the Condition x Session interactions were significant for the nontransplant-enriched ($F(10,160)=4.66$, $P<0.001$), nontransplant-standard ($F(10,170)=6.98$, $P<0.001$) and transplant enriched groups ($F(10,150)=2.36$, $P<0.025$) but not for the transplant-standard group ($F(10,180)=1.09$, $P>0.1$).

While the significant transplant/nontransplant difference, as a whole, does not hold up when examined across subsequent sessions ($F<1.0$, $P>0.1$), there was a significant Transplant x Session interaction ($F(10,260)=3.56$, $P<0.001$) within which analysis of the simple main effects revealed that the transplanted animals had significantly lower weighted-error scores, ($F(1,260)=5.00$, $P<0.05$). By contrast, the enriched groups, across sessions 2-12, had significantly lower weighted-error scores than the standard groups ($F(1,26)=7.32$, $P<0.025$) but there was no significant Environment x Session interaction ($F(10,260)=3.56$, $P>0.1$). No other interaction was significant, Transplant x Environment ($F<1.0$, $P>0.1$) and Transplant x Environment x Session ($F<1.0$, $P>0.1$). However there was a significant reduction in the weighted-error scores across sessions for the lesion groups as a whole ($F(10,260)=6.13$, $P<0.001$).

3.2.7 Error Frequency

Initial Postransplant Performance: The lesion groups made significantly more errors than the control group (Figure 18, left panel). Controls compared to: nontransplant-enriched $t=19.27$ with 16 df, nontransplant-standard $t=7.95$ with 17 df, transplant enriched $t=5.18$ with

15 df and transplant-standard t=9.90 with 18 df, P<0.001 for all tests. During the initial postransplant test session the transplanted rats made significantly less errors than the nontransplanted rats (F(1,26)=5.58, P<0.025). However there was no significant Environment effect (F<1.0, P>0.1) nor a significant Transplant x Environment interaction (F<1.0, P>0.1).

Subsequent Postransplant Performance: Throughout all the subsequent postransplant sessions all the lesion groups made significantly more errors than controls (Figure 18, right panel) Controls compared to: nontransplant-enriched (F(1,160)=69.52, P<0.001), nontransplant-standard (F(1,170)=61.86), P<0.001, transplant enriched (F(1,150)=87.28, P<0.001) and transplant-standard (F(1,180)=108.7, P<0.001). Furthermore the Condition x Session interactions were significant for the nontransplant-enriched (F(10,160)=5.99, P<0.001) and nontransplant-standard (F(10,170)=7.07, P<0.001 groups, but were not significant for the transplant enriched group (F(10,150)=1.33, P>0.1) and the transplant-standard (F<1.0, P>0.1) group.

As a whole the lesioned animals made less errors over sessions (F(10,260)=6.69, P<0.001), furthermore there was a significant Transplant x Session interaction (F(10,260)=4.46, P<0.001), the analysis of the simple effects within it revealed that the transplanted animals made significantly less errors than the nontransplanted animals early on (at sessions 2 & 3 (F(1,260)=6.72, P<0.25) and (F(1,260)=6.91, P<0.025), respectively) thereafter there was no significant difference between the transplanted and nontransplanted groups. Furthermore while the nontransplanted animals experienced a decrease in error performance over session (F(10,260)=10.02, P<0.001) the transplanted animals did not (F(10,260)=1.38, P>0.1). There was no main effect for either Transplants

(F<1.0, P>0.1) or Environment (F(1,26)=2.87, P>0.1) nor was there a Transplant x Environment interaction (F<1.0, P>0.1) a Environment x Session interaction (F<1.0, P>0.1) or a Transplant x Environment x Session interaction (F<1.0, P>0.1).

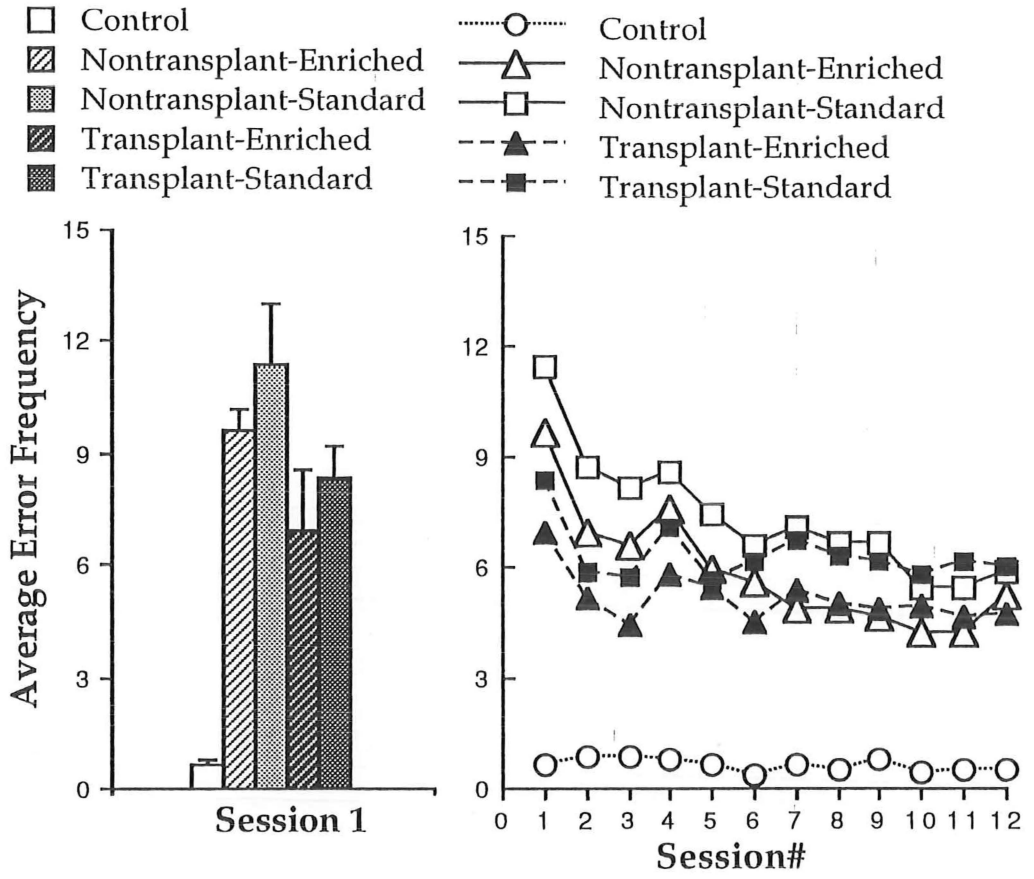

Figure 18. Error Frequency, averaged across groups for each session (Left panel: initial postransplant session, right panel: subsequent postransplant sessions, 2-12). Vertical bars represent the S.E.M.

3.3 Movement (Film) Analysis

Once the areas-under-the-curve had been calculated for each animal, both front and hind paw, the data was subjected to analysis in a similar manner to the beam data. All lesion groups were compared with controls for all 3 sessions with a t-test. All lesion groups were compared to each other with a 2 x 2 x 2 ANOVA (Transplant x Environment x

Sessions, with repeated measures on the last factor) for the 2 postransplant sessions.

3.3.1 HIND PAW

Lesion Group Compared to the Control Group: There was no significant difference between any of the lesion groups and the control group for both film sessions 2 (immediately after the initial postransplant session) and 3 (between test session 11 and 12). Film session 2: Controls compared to nontransplant-enriched, t=0.42 with 16 df, nontransplanted-standard, t=0.33 with 17 df, transplanted-enriched, t=0.44 with 15 df and transplanted-standard, t=0.27 with 18 df, P>0.1 for all t-tests. Film session 3: Controls compared to nontransplant-enriched, t=1.19 with 16 df, nontransplanted-standard, t=1.43 with 17 df (P>0.05), transplanted-enriched, t=0.71 with 15 df and transplanted-standard, t=0.27 with 18 df, P>0.1 for all t-tests (Figure 19).

Comparisons between lesion groups The ANOVA revealed no significant differences between conditions, or their interactions, across both postransplant film sessions: Transplant (F<1.0, P>0.1), Environment, F(1,26)=1,22, P>0.1), Transplant x Environment (F<1.0, P>0.1), Transplant x Session (F<1.0, P>0.1) and Transplant x Environment x Session (F<1.0, P.0.1). Nor did any of the lesion groups, or the control group, display any significant difference over sessions (F(1,26)=5.9, P>0.05) and (F(2,26)=1.013, P>0.1), respectively).

3.3.2 FRONT PAW

Lesion groups compared to the control group: There were no significant differences between any of the lesion groups and the control group at any of the two postransplant film sessions. Film session 2: Controls compared to nontransplant-enriched, t=0.17 with 16 df, nontransplanted-standard, t=1.54 with 17 df (P>0.05), transplanted-enriched, t=0.21 with 15 df and transplanted-standard, t=0.73 with 18 df, P>0.1 for all t-tests. Film session 3: Controls compared to nontransplant-enriched, t=0.52 with 16 df, nontransplanted-standard, t=0.19 with 17 df, transplanted-enriched, t=0.02 with 15 df and transplanted-standard, t=0.25 with 18 df, P>0.1 for all t-tests.

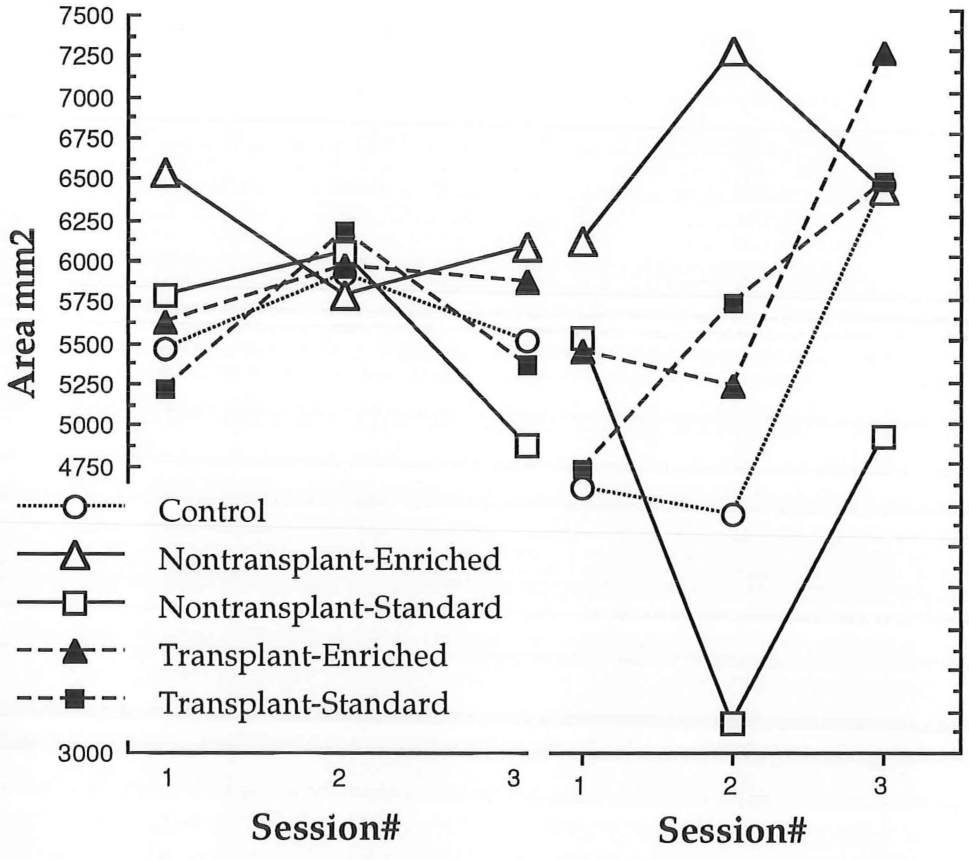

Figure 19. Averages of the area-under-the-curve per sessions, for both the front and hind paw (left and right panel respectively).

Comparisons between lesion groups There were no significant differences between any of the conditions, or their interactions, across both postransplant film sessions (Transplant ($F<1.0$, $P.0.1$), Environment ($F(1,35)=1.876$, $P>0.1$), Transplant x Environment ($F(1,35)=1.58$, $P>0.10$, Session ($F<1.0$, $P>0.1$), Transplant x Session ($F<1.0$, $P>0.1$), Environment x Session ($F<1.0$, $P>0.10$ and Transplant x Environment x Session ($F(1,35)=$ 1.88, $P>0.1$) (Figure 19).

4.0 DISCUSSION

4.1 MAIN FINDINGS AND CONTRIBUTIONS OF THE PRESENT STUDY

4.1.1 SENSORIMOTOR CORTEX LESIONS

Histology revealed that the lesions were appropriate, and though they did not encompass the complete SMC area they included the majority of the critical motor control areas as defined in section 2.3.1. The difference in lesion volumes between the transplanted and nontransplanted animals as seen in Figure 8 was not a statistically reliable one. Furthermore the rats were assigned into their different groups after the lesion surgery, and thus any difference was most likely due to the presence of a transplant and not biased lesion surgery. The fact that lesion size was also not significantly correlated with behaviour means that any transplants influence on lesion size did not induce enhanced recovery of function by shrinking/sparing the lesion.

The present study's results were consistent with several others (Gentile et al, 1978, Slavin et al, 1988, Held et al, 1985, Feeney et al, 1982, Davis et al, 1978, Goldstein et al, 1990 and Swenson et al, 1989) as it has shown that the behaviour of beam running and its associated measures are sensitive to sensorimotor cortex lesions, and are therefore appropriate for use in studies seeking to model such damage. The lesioned animals were (initially) greatly impaired on the locomotor task. The lesion groups ran significantly slower than controls, at the initial

postransplant session, and all lesion groups were significantly impaired to controls on all error types across the total test period.

Furthermore the present study has confirmed that a degree of functional recovery occurs with time and experience after surgery (Kolb, Reynolds and Fantie, 1988 Dunnett et al, 1987). All lesion groups demonstrated a degree of improved performance over testing independently of any other manipulations. However, such recovery was incomplete as while all lesion groups attained parity with controls on run time they did not ever do so for foot errors.

4.1.2 TRANSPLANTS

To the knowledge of the author this study is the first of its kind, in that it attempted to examine the behavioural consequences of the combination of transplant and enriched environment in the context of sensorimotor cortex injury. The present study provides further evidence for the contention that foetal cortex transplants grafted into the injured cortex are capable of surviving and producing a degree of enhanced functional recovery. The transplanted animals made significantly fewer errors at the first postransplant session and across sessions 2-12 than the nontransplanted animals and had a significantly lower weighted-error scores at the first postransplant session and across session 2-12. Furthermore the transplant also promoted recovery to a greater extent than the enriched environments in that their was no difference between the error frequency of the enriched and standard groups, but the transplant group made significantly less errors than the nontransplant group. However, while transplants did promote recovery of function and transplant survival was very high (14 of 15 grafts) the transplant benefit

was limited in the extent to which it enhanced recovery. The transplants conferred an early benefit, after grafting and early on in the behavioural test period, transplanted animals were less impaired during the first 3 to 4 sessions after transplanting compared to the nontransplanted animals, but thereafter there was no difference. This is similar to Dunnett et al (1987) who found that neocortical E21-22 transplants resulted in 'an amelioration of delayed alteration shortly after transplantation'. However the early benefit provided by the transplants in the present study often meant that the transplanted animals reached their maximum recovery early on in the test sessions and experienced no further improvement thereafter, whereas the nontransplanted animals were initially more impaired but they continued to improve throughout the test sessions, reaching parity with (but never becoming significantly less impaired than) the transplanted animals around session 4.

Although the present study found transplant induced functional amelioration of a motor behaviour impairment previous work, by Slavin et al (1988) and Swenson (1989) which also examined the beam task, reported no improvement ascribable to the transplants they used. An exception was Slavin et al's finding that GM1 gangliosides in combination with E19 frontal cortex transplants reduce the behavioural impairment resulting from SMC a lesion. These contradictory results are likely due to a number of design variations between Slavin et al, Swenson et al and the present study. While Slavin et al used a bilateral SMC lesion and observed no transplant induced recovery both the present study and Swenson et al used a unilateral SMC lesion, but Swenson et al did not obtain a transplant benefit whereas the present study did. An important difference between Swenson et al and the present study was the specific tissue taken for grafting, Swenson et al took presumptive SMC tissue while the present

study took frontal pole tissue in the expectation that the frontal cortex's rich supply of trophic substances would promote recovery. Slavin et al also transplanted frontal cortex tissue into the lesioned SMC but did not obtain a transplant benefit, possibly because they obtained poor transplant survival (30-40%) and no functional benefit without the addition of GM1 gangliosides, while the present study obtained both good survival (93%) and a functional transplant benefit. Although Swenson et al also obtained good survival (92%) they did not obtain a functional transplant benefit, possibly because they used young foetal material (E14-15) which may have promoted transplant survival but not functional recovery.

The present study transplanted 30 days after lesioning and obtained both good survival and recovery, whereas Slavin et al (1988) transplanted with a short delay period (7 days) and obtained neither survival nor recovery and Swenson et al (1989) transplanted immediately after lesion surgery and obtained good survival but no recovery. One explanation for the disparity between Slavin et al, Swenson et al and the present study is that the present study employed suspension transplants while the others employed solid tissue. This difference is especially relevant in light of transplant survival as Slavin et al reported that their poor survival (30-40%) was very likely due to insufficient vascularization, while a suspension transplant is immediately exposed to vascularization and thus the nutrients it requires. As mentioned the point that Swenson et al transplanted solid tissue yet obtained high survival is probably due to the fact that they employed young foetal material grafted into the neonatal (and therefore rich in trophic substances) frontal cortex. The fact that the present study employed a postlesion-pretransplant delay of 30 days and still obtained excellent transplant survival and a degree of

behavioural recovery is quite contradictory to that which Cotman et al (1985), Lescaudron and Stein (1990) and Sandor et al (1991), would have predicted, and especially Kimble (1990) who stated that suspension grafts 'usually require earlier donor ages than do solid tissue grafts'. However, as discussed in the introduction, there is already a reasonable degree of contradictory evidence as regards the relevance of the delay window, especially for transplant survival, and this study is simply another example of such evidence.

The fact that what transplant enhanced recovery of function was observed was largely seen early in the course of testing suggests that the functional transplant benefit is very unlikely to be due to reconnection of neurons as insufficient time (transplant induced recovery of function was observed within 14 days of transplanting) had passed for that to occur. Also the fact that the transplant was made into the parenchyma surrounding the lesion site further supports the contention that reinnervation was not responsible for the transplant induced benefit as such a suspension transplant would be unlikely to induce a beneficial influence by acting as a bridge to reconnect severed structures. Thus the incidence of survival and enhanced recovery of function demonstrated in this study must have been produced by some other mechanism than neural reinnervation, with the most likely contender being the 'trophic pump' hypothesis. If so it is likely that the graft derived trophic factors promoted transplant survival, possibly by encouraging transplant growth and the development of a nutritive infrastructure with the host, and assisted recovery of function, perhaps by reducing secondary degeneration and/or by enhancing the plasticity of damaged regions. The high degree of transplant survival was probably aided by the fact that the graft was made of frontal cortex material. As discussed in the

introduction, such material is rich in trophic factors which would very likely have helped to make up for the lack of host derived trophic support due to the long delay. The fact that a transplant made of frontal cortex material was transplanted in a heterotopic manner and demonstrated a very good degree of survival plus a degree of enhanced recovery of function is further evidence for the contention that transplants of frontal cortex material are capable of surviving in heterotopic areas and are capable of enhancing functional recovery of function from damage to the SMC. As it is extremely unlikely that heterotopic material would be capable of producing appropriate reinnervation (Lescaudron and Stein, 1990 and Cassel et al, 1992) it seems very likely that the high level of transplant survival and the transplant induced functional recovery present in the current study were influenced by the trophic support presumed to be supplied by the frontal cortex transplant. Which is consistent with the conclusions drawn by those studies that have transplanted frontal cortex tissue in a heteretopic manner, Lescaudron and Stein found that transplanting frontal cortex tissue into the lesioned occipital cortex was better at promoting recovery of a brightness discrimination task than occipital cortex transplants, and Sandor et al (1991) found that frontal cortex transplants grafted into the lesioned SMC functioned in 'a way analogous to [the] normal cortex'.

Sandor et al (1991), Plumet et al (1991) and Soares et al (1991) also obtained transplant induced functional recovery of impaired motor behaviour, but they employed different behavioural tasks (lever pressing, paw reaching and general motor behaviour, respectively). While Sandor et al transplanted frontal cortex tissue into the unilaterally lesioned SMC and obtained 100% transplant survival and graft behaviour 'analogous with the normal SMC'[check quote] they employed neonatal subjects and

grafted E17-18 tissue 7 days after lesioning. Plumet et al also obtained 100% survival and partial amelioration of functional impairment but transplanted E16 frontal cortex tissue into the unilaterally lesioned frontal cortex. Soares et al transplanted E16 lateral parietal cortex into the unilaterally lesioned lateral parietal cortex after a variety of delays and observed some improved general motor behaviour in the 7 day delay subjects. The fact that those studies that were not as similar to the present design as Slavin et al (1988) and Swenson et al (1989) obtained transplant induced amelioration of an impaired motor behaviour while the Slavin et al and Swenson et al did not suggests that frontal cortex transplants are generally beneficial for impaired motor function (Soares et al also employed frontal cortex transplants, but in a homotopic manner) and that it is the origin of the transplant that determines whether or not it will induce recovery of function from cortical injury. However the fact that a specific description of where the transplant is taken from is often missing makes it difficult to draw firm conclusions about the benefit, or otherwise, of grafts made from specific regions.

4.1.3 Enriched Environment

As predicted, the enriched environment produced an enhanced degree of recovery of function as measured on the beam task. This is consistent with the literature and is further evidence that an enriched postoperative environment is beneficial for recovery from sensorimotor cortex injury. The enriched environment animals, regardless of transplant status, were improved in comparison to the standard environment animals in terms of Error types 3, 4 & the weighted-error-total. This improvement was demonstrated early in the test period, but unlike the transplanted animals the enriched animals continued to show

an improvement throughout the course of testing on these measures. Furthermore the enriched animals were not impaired in comparison to the standard animals but unlike the nontransplanted animals, who reached parity with the transplanted animals after the early sessions, the standard animals never reached parity with the enriched animals throughout the subsequent test sessions. However there was no difference between enriched and standard animals as measured by run time, error types 1 & 2 and error frequency and the enriched rats remained impaired in comparison to controls. Thus while the enriched rats in the present study were less impaired than the standard rats they did not perform as well as the enriched rats reported in Held (1988) and Kolb and Gibb (1991, in Will and Kelche, 1992). Held et al reported that their impoverished/enriched rats (analogous with the enriched rats in the present study) quickly reached parity with the control group as measured by run time, just as the enriched rats in the present study did, but Held et al also report that their impoverished/enriched rats ran significantly faster than their impoverished/impoverished rats (analogous with the present study's standard rats) whereas there was no difference between the enriched and standard animals as measured by run time in the present study. (Held et al did not measure foot faults). Kolb and Gibb reported that their enriched rats did not make significantly more foot errors than controls after a unilateral or bilateral 'frontal' lesion and that their impoverished (standard housing) rats were always impaired in comparison to controls, whereas the present study found that <u>both</u> the enriched and standard housing rats were always impaired in comparison to control rats as measured by foot faults. Furthermore the enriched rats in the present study made <u>more</u> minor foot faults than the impoverished animals at the initial postransplant session However it is worth noting that Held et al's enriched environment included wide ramps and 'elevated platforms'

which, while not being the actual beam task provided the rats with the ability to practise locomotor behaviour along a beam, especially as the environment ramps and test beam were very similar in width (7.5cms and 5cms respectively). This point is particularly relevant in light of the fact that simply allowing a lesioned animal the ability to practise an impaired behaviour promotes recovery (Feeney et al, 1982) as the rats performance improved dramatically after experience on the beam during the pre-surgery training period. While author was unable to obtain any information about the objects within the enriched environment employed by Kolb and Gibb it should be noted that they employ a 'frontal cortex' lesion which may not damage as much of the SMC as in the present study and therefore their enriched rats may not have had as much of an impairment to begin with. Also this study deliberately avoided using junk objects in the enriched cages that would allow the rats to practise beam running and the present study only changed the junk objects twice a week. While this was consistent with Dunnett et al (1986) the convention is to change the enriched environment objects more often, thus the present studies enriched rats may not have gained as much of a benefit from their enriched environments as was possible and for this may explain why they did not perform as well as other enriched rats. However the present studies enriched rats did perform better than their impoverished counterparts and their behaviour improved in a manner that was consistent with some of the relevant literature, Held et al (1985) reported that their impoverished/enriched animals (being analogous with the nontransplant-enriched animals in the present study) were not significantly slower than their controls after the first 2 postransplant sessions, which is similar to the present study in that the lesioned animals had slower running times than the control animals at the initial

postransplant session only, and thereafter the lesion groups were not significantly slower, regardless of housing status.

4.1.4 COMBINATION TREATMENTS

What little literature that exists on combining transplants with enriched environments suggests that doing so would produce amelioration of function over and above of that of either the two treatments in isolation. However the results of the present study do not support this. While the transplanted-enriched group displayed the early benefit idiosyncratic of the transplant groups it did not display an interactive benefit, as after the first 3-4 postransplant sessions it did not perform better than the nontransplanted-enriched environment group.

The two studies that have previously examined the combination of environment and transplant have reported an interactive benefit for those animals which combine the two treatments (Kelche et al, 1987 and Dunnett et al, 1986). However it must be noted that those studies varied from the present one in some very important ways. First, Dunnett et al transplanted basal forebrain tissue into the 'dorsolateral frontal cortex', and assessed histological features of the grafts, not the behavioural consequences of the combination of transplants and enriched postoperative environment. Dunnett et al also reported no significant difference between groups for graft volume and the present study also found that graft volume was unaffected by housing condition (at 7 weeks after grafting). Second, the Kelche et al study transplanted foetal basal forebrain into the lesioned Fimbria-Fornix and examined Hebb-Williams maze learning, whereas the present study transplanted frontal cortex material into a SMC lesion and examined locomotor performance.

The conclusion of the present study is that both transplants and environments are capable of enhancing recovery of function independently of each other but that an enriched environment combined with transplants did not interact to produce a greater degree of enhancement. This suggests that the damage produced by sensorimotor cortex lesions is capable of being ameliorated to a certain degree only, whether the mechanism of amelioration is a transplant or an enriched environment. Except that the specific form of recovery varies slightly for the two therapies in that a transplant promotes recovery sooner after grafting than exposure to an enriched environment and reduces error frequency and that while transplant induced recovery plateaus within 3 weeks of grafting an enriched environment continues to improve motor performance for up to 8 weeks of testing, though not to the extent that it was significantly better than the transplant group.

4.2 LIMITATIONS OF THE PRESENT STUDY

One limitation of the present study is the results of the film analyses which were not consistent with the literature. The analysis failed to demonstrate any lesion induced deficit and as therefore was unable to demonstrate any amelioration, or otherwise, of the deficit. One problematic aspect was that the motor patterns revealed by the film analysis were similar to the aberrant movement patterns reported in the literature. Held et al (1985) noted that their 'impoverished/impoverished' animals displayed an aberrant motor pattern that was characterised by a very low initial flexion phase and examination of the motor patterns (Figure 19) shows that such a phenomenon was present in all groups at session one (prelesion), and in the control group throughout all film sessions, which suggests that the rats were forced to adopt an atypical

movement pattern when running on the very narrow beam. One reason why the subjects in the present study displayed an aberrant motor pattern in comparison to the motor patterns reported by Held et al, (1985), Gentile et al, (1978) and Slavin et al (1988) may be because those authors used a 5cm wide beam whereas the beam used in the present study was only 2.5cm wide. It seems probable that the extremely narrow beam used in this study forced the animals to adopt an atypical motor pattern and that this pattern was more variable over runs, subjects and sessions.

Had the author analysed more areas-under-the-curve for each animal the within group variability would have been lowered and a lesion influence may have emerged. Both Gentile et al (1978) and Held et al (1985) examined 6 areas-under-the-curve for each animal. However doing so would not have necessarily resulted in movement patterns similar to those found by Held et al and Gentile et al and the movement patterns may still have been atypical in comparison to those found in the literature.

4.3 RECOMMENDATIONS FOR FUTURE RESEARCH.

The findings of the present study support the value of frontal cortex suspension transplants grafted into the lesioned cortex, but future studies would add to this area of research by employing foetal tissue of different ages and/or different postlesion delays and/or a solid transplant. This would help to establish whether or not the recovery produced in this study was a phenomenon of the relatively old tissue and/or the long postlesion delay and/or the suspension graft. The issue of solid or suspension transplants is especially relevant in light of the

author's claim that it was the use of a suspension transplant into the parenchyma that produced the transplant benefits observed in this study.

It would be useful to extend the postransplant period as the present study delayed for only 8 weeks postransplant and is therefore incapable of providing evidence to show whether or not frontal cortex suspension transplants are capable of surviving for longer periods and whether or not such survival would benefit or impair recovery. It would also be useful to examine the brains for secondary damage, in order to determine if the transplant derived trophic factors spared such damage and/or promoted neurological plasticity as was suggested earlier. An attempt was made to do so in the present study in that thin sections (25μl) of the animals thalamus were mounted and stained with cresyl violet in preparation for histological investigation but the their analysis was beyond the scope of the present thesis.

A replication of the present study employing beams of different widths would help to explain the difficulties encountered in this study as regards the film analysis. If it was the particularly narrow beam that was responsible for the difficulties associated with the film analysis then it would be reasonable to employ a wider beam (5cm.) in future studies concerned with film analysis of movement patterns. However, while the narrow beam may interfere with the movement topology, it is capable of demonstrating any functional impairment resulting from SMC lesions, and any recovery from such an impairment.

Another study examining a transplants effect in rats with SMC lesions would benefit from employing additional measures that would allow a more thorough assessment of the behavioural influences of

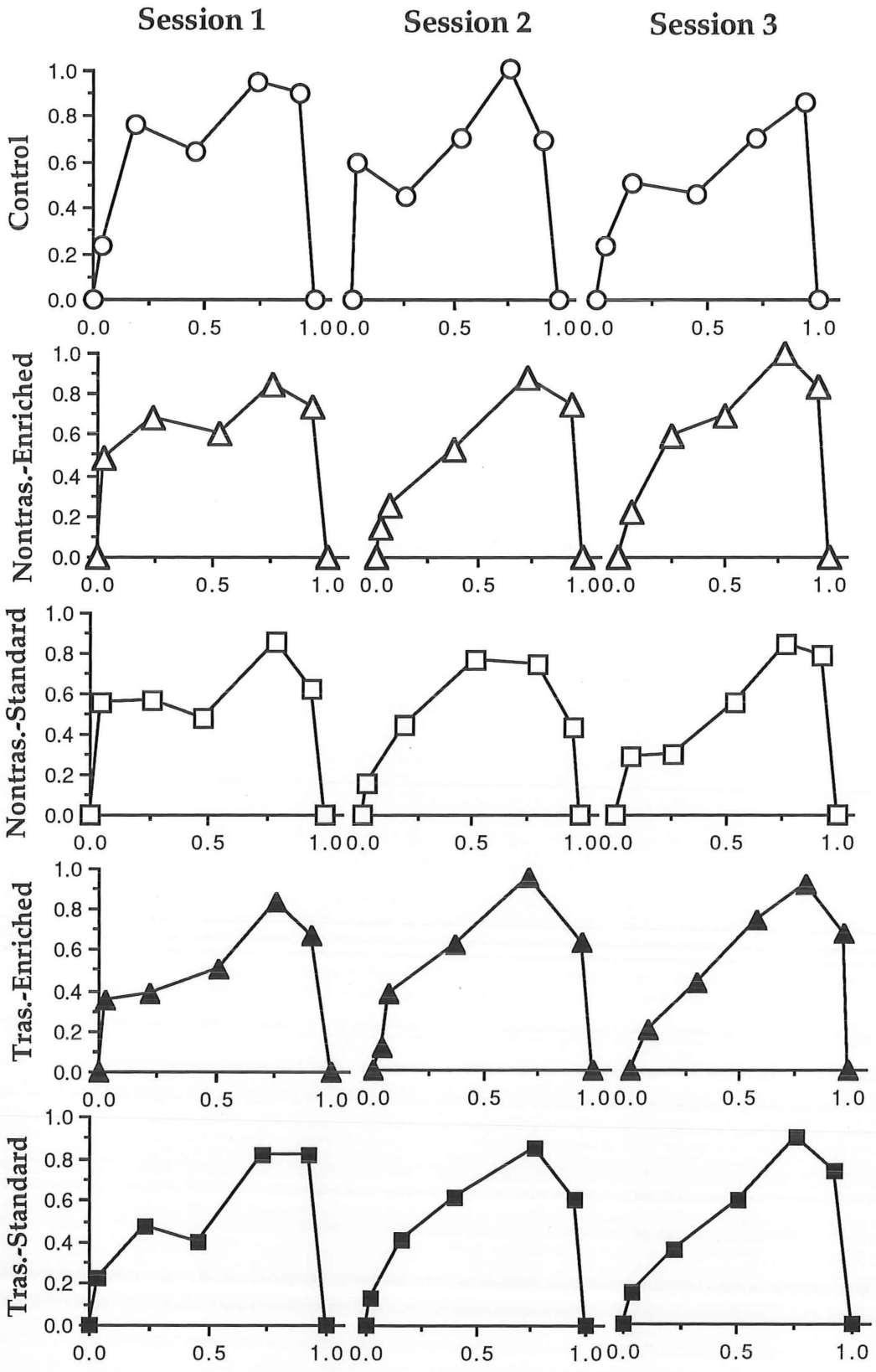

Figure 20. Normalized hindlimb movement patterns for film sessions one, filmed immediately prior to lesion surgery, two, filmed between postransplant test sessions 1 & 2, and three, filmed between postransplant test sessions 11 & 12.

transplants and enriched environments. For instance some task that focused upon manual dexterity of the front paw would be useful, especially in light of the fact that while the front paw motor control area was lesioned the majority of the behaviours analysed in this study were drawn from observations of the hind paw. One possibility would be to employ a paw reaching task as per Plumet et al (1991).

While this study did not demonstrate any interactive benefit of transplants and enriched environments the issue is far from decided and further examination of the two therapies in combination is warranted. As the combination of transplants and enriched environment did not produce greater recovery than either therapy individually it seems likely that recovery (as observed in the present study) was produced by addition of the two factors rather than interaction. Recovery as a result of interaction may well result in greater amelioration than that produced by addition, and is therefore desirable.

4.4 Concluding Comments

The failure of this study to demonstrate an interactive effect for transplants and enriched environment is disappointing. However the clear demonstration of both a simple transplant and environment benefit is useful. Moreover the demonstration that transplants are capable of not only surviving in the damaged cortex, but also are capable of partially ameliorating the functional impairment resulting from sensorimotor cortex injury is very encouraging. Furthermore the suggestion that the critical postlesion delay period is not crucial to obtaining functional recovery is valuable, particularly as it would not always be possible to fulfil this conditions in terms of applying transplant therapy to humans

with sensorimotor cortex injury. The likelihood that the recovery produced in this study was due to the preparation and origin of the transplants is also interesting, as it provides strong evidence for the contention that transplant induced recovery of function, in the cortex, is due to the trophic support provided by the donor tissue, as opposed to trophic support provided by both the host and donor tissue and/or the re-establishment of appropriate neural connections.

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